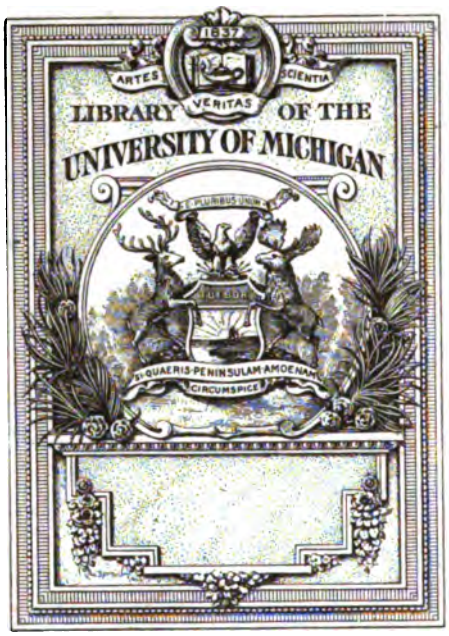

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Preface

This volume of publications from the Wistar Institute is intended to present in a brief manner the activity of the Institute during the year 1907. Twelve papers, here presented, were published in 1907, two in December 1906 and two in 1905.

An examination of these papers, beginning with Bulletin No. 1, will throw some light upon the progress of the Institute without the necessity of further explanation. As will be observed, the Institute does not publish its researches in any single journal, but distributes them in the various departmental journals according to the subject matter. In this manner the most satisfactory publication is obtained. Only a few copies of this bound volume were issued, and it is not intended as a part of an Institute series, as the separate articles are always to be found in their respective journals.

The Wistar Institute is, I believe, the first university institute in America devoted exclusively to researches in Anatomy and Biology. Its origin is due to the broad minded policy of the University of Pennsylvania and to the liberality of the late General Isaac J. Wistar. Its object is to maintain a museum and laboratories for the promotion of advanced studies and researches in Anatomy and Biology. It is National in scope. Its laboratories are open alike to properly qualified students from all institutions.

Milton J. Greenman

Director

BULLETIN

of the

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

No. 1.

September, 1905.

In 1892 a charter was secured by General Isaac J. Wistar, of Philadelphia, from and under the laws of the Commonwealth of Pennsylvania, conferring perpetual incorporation, with the right of perpetual succession and a corporate seal, upon a corporation to be called The Wistar Institute of Anatomy and Biology.

A modern fireproof building costing $125,000, was erected at Thirty-sixth Street and Woodland Avenue, in the city of Philadelphia, upon land donated by the University of Pennsylvania, and an endowment sufficient to yield an annual income of $3000, was vested by General Wistar in a trustee. The Institute was formally opened on May 21, 1894.

The principal objects of the Institute, as stated in its charter, are sheltering, preserving and increasing the extent and usefulness of the anatomical museum originally instituted by Dr. Caspar Wistar, and the promotion of advanced study along Biological lines. The nucleus of the Institute's anatomical museum was the first collection of the kind in America, begun by Dr. Caspar Wistar, while Professor of Anatomy in the University of Pennsylvania, 1808-1818. After his death it was presented by his widow, Elizabeth Mifflin Wistar, to the University of Pennsylvania, and the University of Pennsylvania presented it to the Wistar Institute.

The first building of the Institute, erected in 1893, is approximately 220x67 feet, and four stories high. In 1897 there was erected a new wing, 72x46 feet, and of the same height. In the basement of this wing are located the heating and lighting plants.

About one-half of the entire building is used as a museum. The other half is devoted to laboratory purposes. During the interval between the opening of the Institute in 1894 and the present year, 1905, the endowment has been increased by the same donor until the annual income is approximately $40,000. Of this about one-half is each year added to a reserve fund. By the will of General Isaac J. Wistar, who died September 18, 1905, the Wistar Institute becomes residuary legatee to his estate. During this interval also, under the successive directorships of Dr. Harrison Allen and Dr. Horace Jayne the collection in the lines of human and comparative anatomy has grown from some 3000 objects to more than 14,000; the value of the collection having increased in even greater proportion.

A good working library has been accumulated and the laboratories are equipped with modern apparatus. In accomplishing these results the Institute has been materially assisted by special contributions from those interested in the work.

In January, 1905, Dr. Milton J. Greenman was elected Director. The following April a Conference of American Anatomists was called at the Institute for the purpose of considering its future policy.

This conference made the following recommendations, which were presented to the Institute:

1. The principal object of the Institute to be research. This would involve:
   (a) the appointment of a chief of investigation in one or more fields;
   (b) the appointment of research assistants, as well as men who shall divide their services between the museum proper and research;
   (c) the appointment of technical assistants.
2. Research. The research shall be:
   (a) in the field of neurology,
   (b) comparative anatomy and embryology.
3. Relations. The committee recommends:
   (a) that a subvention to the Journal of Anatomy be granted;
   (b) that a committee be appointed to consider the relations of the Wistar Institute to American anatomists;
   (c) that the Wistar Institute apply to the Association of American Anatomists for co-operation.
4. The committee recommends that an Advisory Board of ten be appointed for general purposes:
   (a) to form a sub-committee on neurology, as well as other sub-committees that may be needed;
   (b) to establish relations with the committee of the International Association of Academies for Brain Investigation and with other committees for collective investigation.
5. The committee would further state that while the general trend of work above outlined is recommended for the present, there is no intention to advise a limitation of the activities of the Institute to it exclusively.

In carrying out the general policy proposed at this conference the Wistar Institute created an Advisory Board of Anatomists and elected the following anatomists as members:

Dr. Lewellys F. Barker, Professor of Medicine, Johns Hopkins University, Baltimore.

Dr. Edwin G. Conklin, Professor of Zoology, University of Pennsylvania, Philadelphia.

Dr. Henry H. Donaldson, Professor of Neurology, University of Chicago, Chicago, Ill.

Mr. Simon H. Gage, Professor of Histology and Embryology, Cornell University, Ithaca, N. Y.

Dr. G. Carl Huber, Professor of Histology and Embryology, University of Michigan, Ann Arbor, Mich.

Dr. George S. Huntington, Professor of Anatomy, Columbia University, New York.

Dr. Franklin P. Mall, Professor of Anatomy, Johns Hopkins University, Baltimore, Md.

Dr. J. Playfair McMurrich, Professor of Anatomy, University of Michigan, Ann Arbor, Mich.

Dr. Charles S. Minot, Professor of Histology and Human Embryology, Harvard Medical School, Boston, Mass.

Dr. George A. Piersol, Professor of Anatomy, University of Pennsylvania, Philadelphia, Pa.

The Advisory Board organized by electing Dr. Charles S. Minot chairman and Dr. Milton J. Greenman permanent secretary. It then proceeded to appoint the following committees:

(1) On Neurology and the Establishment of Relations with the International Association of Academies, Dr. Lewellys F. Barker, Dr. Henry H. Donaldson, Dr. Franklin P. Mall, Dr. J. Playfair McMurrich, Dr. Charles S. Minot. This committee to elect its own chairman.

(2) On Relations of the Wistar Institute to American Anatomists, Professor Simon H. Gage, chairman; Dr. George A. Piersol, Dr. G. Carl Huber.

(3) On Comparative Anatomy and Embryology, Dr. George S. Huntington, chairman; Dr. Edwin G. Conklin, Dr. Franklin P. Mall.

In pursuing the plan further Dr. Henry H. Donaldson was elected Professor of Neurology, and Dr. ———— ————, Associate in Neurology in the Wistar Institute of Anatomy. Dr. Donaldson will assume charge of the neurological work of the Institute on October 1, 1905. Other appointments are now being considered for the purpose of organizing a strong staff of neurological research workers.

It is desired to make the Institute a central station for anatomical research in this country, and for this purpose to co-operate in every way possible with University work in this field, and while it is proposed, for the time being, to direct the energies of the Institute largely to problems in neurology, opportunities for work will always be granted to investigators interested in other divisions of anatomy.

Three members of the Advisory Board, namely Dr. Donaldson, Dr. Mall and Dr. Minot, are members of the Central Commission of the International Association of Academies for Brain Investigation, thus placing the Institute in close touch with the work abroad. It is hoped that the institutions of learning of the country may co-operate with the Wistar Institute in promoting the science of anatomy by recommending to its laboratories men who will aid in the advancement of knowledge in this department of science. At the same time it is not essential that persons should come from other institutions, as the Institute is open to any capable investigator who is prepared to make use of the advantages which it offers.

At present the Institute does not undertake any independent publications, but will utilize the existing scientific journals.

The Institute will act as conservator of series of specimens or other material already studied which should be preserved for future reference, and which may for this reason be presented to the Institute.

It will send out to investigators, in other places, materials for research work which it may have in its museum or collect and prepare such material whenever this is possible.

The reproduction of new models when they are of great value for teaching will also be undertaken.

The Wistar Institute is controlled by a Board of Managers consisting of the following gentlemen:

Mr. Arthur E. Brown, Mr. Samuel Dickson, Dr. Samuel G. Dixon, Mr. Joseph S. Harris, Mr. Charles C. Harrison, Dr. Robert G. Le Conte, Dr. Morris J. Lewis, Dr. S. Weir Mitchell, General Isaac J. Wistar.

Mr. Charles C. Harrison, president; General Isaac J. Wistar, secretary†; Mr. Henry G. Brengle, treasurer.

The Advisory Board of Anatomists chosen to advise as to the scientific work of the Institute, consists of the gentlemen above named.

The resident scientific staff consists of the following members:

Dr. Milton J. Greenman, Director.

Dr. Henry H. Donaldson, Professor of Neurology.

——— ———, Associate in Neurology.

Dr. J. Macpherson Stotsenburg, Junior Associate in Anatomy.

Dr. Harold D. Senior, Junior Associate in Anatomy.

Communications relative to the work of the Institute may be addressed to any member of the Advisory Board or to the Director at the Wistar Institute, corner Thirty-sixth street and Woodland avenue, Philadelphia.

Note.—The Associate in Neurology has not yet been appointed.

A COMPARISON OF THE WHITE RAT WITH MAN IN RESPECT TO THE GROWTH OF THE ENTIRE BODY

BY

HENRY H. DONALDSON

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A COMPARISON OF THE WHITE RAT WITH MAN IN RESPECT TO THE GROWTH OF THE ENTIRE BODY.

BY HENRY H. DONALDSON

In collaboration with ELIZABETH HOPKINS DUNN and JOHN B. WATSON.[1]

IN order to give greater value to a further study of the growth of the nervous system of the rat, it was thought necessary to establish the general growth-relationships between the two forms mentioned in the title. We propose, therefore, in this paper, to compare in the white rat and man the manner in which the body gains weight between conception and maturity.

The white rat used for this study is distinctly smaller and more lightly built than the brown rat (*Mus decumanus*), and hence all the measurements assigned to it are somewhat less than those of the brown species with which we are familiar. It is commonly stated that the white rats kept as pets are albinos of the black rat (*Mus rattus*). This statement is certainly not true for the colony on which our observations have been made. At the moment more cannot be said; but investigations now in progress for the purpose of establishing the zoölogical relationships of the white rat probably will enable us at an early date to make additional detailed statements concerning our own colony.

In carrying out a comparison between forms so dissimilar in absolute size as the rat and man, it is of course impracticable to employ the same scale in the charts intended to exhibit the relations. Under these circumstances, two adjustments have been made in the method of recording, so as to render the results more directly comparable.

In the first place, the base-line, on which are plotted all the

[1] From the Neurological Laboratories of the University of Chicago and of The Wistar Institute of Anatomy, Philadelphia.

curves for change according to age, is of the *same* length for both forms. This means that we make the period representing the span of life in the rat extend on the chart over as long a distance as the corresponding span in man. The first difficulty met in attempting to do this accurately lies in our incomplete information concerning the onset of old age and the natural length of life in the case of the rat. From the best data which we can obtain, we have, however, concluded that the three-year-old white rat is very old, and is justly comparable to a man of ninety years. For the present purpose, therefore, we call the span of life in the white rat three years, and compare this with ninety years of human life. It is assumed that the same proportional relations can be used for fractions of the entire span of life. In this study our interest lies for the most part in the first year of rat life, corresponding, in accordance with the above relation, to the first thirty years of human life.

In the second place, the reduction of the values of the ordinates, so as to bring the two curves together at their termini, is a simple matter of adjustment, the details of which will be given in their proper place.

Data on the White Rat.

The material on which we depend for the body-weight of the white rat at different ages has been obtained from records made by Dr. Dunn and Dr. Watson, and from several other series preserved in the archives of the laboratory (see Tables I and II).

Dr. Dunn's observations touch the first fourteen days of life, and need a word of introduction and explanation.

Dr. Dunn's Series. — After birth the young white rat depends upon the mother for sustenance for about twenty days. Previous experiments had shown that during this period of dependence, especially during the first half of it, removing the young rats from the mother to weigh them resulted in checking their growth. The causes of this retardation are not far to seek, but they need not be detailed now.

We decided, therefore, to obtain the weight of the rats during the first fourteen days of life by weighing different litters

as they attained different ages, and thus weighing each litter only once. Since individuals taken from different litters often exhibit much greater differences in weight than is usually shown by the extremes of a single litter, it follows that by this method the extreme values at any age are somewhat greater than they would be, could the same litter have been followed through successive stages.

The make-up of a litter is always unpredictable, and ranges from those consisting entirely of representatives of one sex to those evenly divided between the two. However, in Dr. Dunn's work, no litter was weighed which did not have at least four individuals in it, and none with less than two of the same sex. Where the representatives of one sex were more numerous than those of the other, the number of the former was reduced by excluding the lightest *and* heaviest representatives, until it equalled the number of the other sex, or differed from that number only by one. Thus throughout there was approximately an equal representation of the two sexes in each litter, —a point of importance when determining the relative growth of the sexes during the first fourteen days. In calculating the body-weights at birth, and at one and two days, there have been added to Dr. Dunn's records those in the laboratory archives. This addition is explained in detail in the note to Table VII.

DR. WATSON'S SERIES. — Beginning with the fifteenth day, the records in the tables (I and II) up to a hundred and eighty-five days for the males, and a hundred and ninety-two days for the females, were obtained from animals carefully reared by Dr. Watson. He began his work with ten litters, nine of which were born in May, and one in June, 1903. From these ten litters there were chosen nineteen males and seventeen females, the effort being made to obtain two of each sex from each litter. The animals to be used were selected when the litters were fifteen days old, and the first weighing was made at that time. At twenty days the chosen animals were weaned; and at about sixty days (i.e., just before the period when sexual maturity is reached) the two sexes were separated, and neither group was allowed to breed. The conditions surrounding these animals during this period of observation were such as have been found

favorable for growth. The food was varied and abundant, but not excessive in amount. The care of the animals was very similar to that described by Dr. WATSON in his article on "The Effect of the Bearing of Young," etc. (1905).

The weighings were made just before feeding. The first record of the weight of the rats was made at fifteen days, and individual records were kept throughout the experiment. From the fifteenth to the thirty-first day the rats were weighed every second day; from the thirty-first to the ninety-second day, every third day; and from the ninety-second day to the hundred and twenty-fourth, every fifth day. From this last age to the end of the experiment they were weighed every seventh day. The rats became quite tame, and towards the end of the experiment would sometimes climb into the scale-pan of their own accord.

These observations, however, were not carried on without disturbance caused by the illness of some of the rats. Suffice it to say on this point, that when the rat, by its behavior, was found to be ill, and to be either falling behind in its rate of growth or actually losing weight, it was excluded from the record. The preceding portion of the record, which fell within the limits of those for the other normal rats, was, however, retained. It thus happened that at the end of the period of observation there were only fifteen males and eleven females which were considered in a normal, healthy condition.

As stated above, neither the males nor the females in Dr. Watson's series were mated. The effect of mating on the growth-curve for the males can probably be neglected, but in the case of the females it is an important circumstance.

Since, as a rule, laboratory animals of this sort are not isolated, it seemed most probable that other investigators would wish to compare the weights of the females which had been mated with the records which we have to present. To meet this possibility a second series of *calculated* weights, based on Dr. Watson's investigation on the effect of the bearing of young, is introduced, showing for those females allowed to bear young the estimated body-weight at different ages after the beginning of the first pregnancy. This is always greater than

the body-weight of the unmated females of like age. In this connection it is desirable to emphasize the fact that under ordinary circumstances the true body-weight of breeding females is difficult to determine. The weights of bearing females should be taken, as Dr. Watson has shown, only between the periods of pregnancy and after the rat has actually recovered from the prolonged strain of nursing the young; and the attention of those who have occasion to record body-weights of females is called to this point.

What we endeavor to show in the second series of numbers in Table II is the body-weight as modified by the bearing of young after the immediate disturbances due to the rearing of the litter have disappeared. In looking over Dr. Watson's records, as given in the paper cited above, it is seen that this cycle of disturbance, from the beginning of pregnancy to the end of the recovery from lactation, comprises from eighty to ninety days. We have assumed, then, that from impregnation to complete recovery would occupy a period of at least eighty days; and we have also assumed that the effects of the bearing of the young, so far as they influence the body-weight of the female, can be here represented as though they were steadily progressive.

In Dr. Watson's experiments on the effects of the bearing of young, the gain in weight extending through an average period of two hundred and forty days, and comprising the final recovery from the last of three litters, was found to be approximately .03 of 1 per cent. (0.03 per cent.) of the initial weight per diem; that is, mated animals increased in body-weight this much more rapidly than those which remained unmated.

Beginning in the present instance with the ninety-second day, at which time the effect of the first litter might be exhibited by a noticeable increase in the weight of the mother, conception having occurred ten days earlier, we have calculated and added the excessive growth of the mated females between the ninety-second day and the hundred and ninety-second day, the time at which this series closes. In doing this, the "initial weight" taken as the basis for the calculations was that at eighty-eight days (average, 136.0 grams: lowest, 115.6 grams; highest,

157.4 grams). The weights thus calculated for the breeding females are those used for the construction of the curve in Plates II and III.

After the hundred and ninety-second day, we have observations which have been collected in the laboratory at different times. They furnish seven cases at about three hundred and sixty-five days, or one year, all of these animals having been allowed to breed under the ordinary laboratory conditions.

In the case of the males, in Dr. Watson's series, no special remarks are called for. His records run only to the hundred and eighty-fifth day, and are continued by laboratory records which fall into four groups:—

10 individuals about 216 days old.
10 individuals about 256 days old.
6 individuals about 365 days old, or 1 year.
6 individuals about 730 days old, or 2 years.

We are thus able to get information concerning the change in body-weight of the female up to one year, and of the male up to two years.

In order to complete the growth-record in the rat, we need to know the changes in weight from the date of conception to that of birth. Unfortunately, it was not until the last moment that the need of this record was appreciated. The data will be gathered, but this will require some time; and for the present we shall use a curve the values of which have been calculated. The basis for this calculation is found in the records of FEHLING (1877), on the growth of the fœtus in the rabbit. The gestation period of the rabbit is from thirty to thirty-one days. This time is divided, in his table, into ten equal periods; and the weights, starting with the beginning of the fifth, are entered for each period. It is assumed by us that the nearly related rat, the young of which are as immature as those of the rabbit, and the gestation period of which is twenty-one days, grows in the same manner as does the rabbit (see Table III).

Under these circumstances, the change in weight of the rat can be approximately estimated; and the part of the curve which represents the increase in body-weight before birth, and

comprises the first two phases of the growth-curve, which are discussed further on, can in this way be provisionally represented.

The method of presenting the results on the body-growth of the rat which are entered in Tables I and II was carefully considered. Each age-group might have been examined statistically, and weight variants determined for it; but it was thought that the value of such results would be hardly enough greater than that given by printing the extreme weights for each age and group to warrant the additional labor and tables. The limiting individual records tend to be aberrant, and hence make a less favorable showing than could be obtained from a more elaborate treatment of the data; but this will hardly mislead any one who wishes to utilize these results. The entire series of individual records is preserved in the archives of The Wistar Institute of Anatomy, and is open to inspection there. All of the data for the rat are given in Tables I and II, and with these it will be necessary to compare the corresponding observations which apply to man.

TABLE I. DATA ON WHITE MALE RATS (UNMATED), SHOWING INCREASE IN WEIGHT OF BODY WITH AGE.

AGE IN DAYS (Gestation 21 Days).	BODY-WEIGHT IN GRAMS.			NUMBER OF ANIMALS.
	Average.	*Lowest.*	*Highest.*	
Birth	5.4	4.3	6.5	40
1	5.6	4.6	6.7	26
2	5.8	5.2	6.3	10
3	6.3	5.6	6.7	8
4	6.9	6.5	7.9	10
5	8.3	7.1	9.6	9
6	9.1	6.7	12.7	11
7	9.2	7.3	12.7	11
8	10.4	7.2	13.1	14
9	11.3	9.1	13.7	10
10	12.2	10.8	13.5	6
11	13.3	13.0	13.6	4
12	14.8	11.4	19.5	6
13	15.3	14.1	16.0	5
14	15.2	14.0	17.6	6
15	16.5	12.5	22.4	19
17	17.8	13.9	24.0	19
19	19.5	15.2	26.0	19
21	21.2	14.6	30.1	19
23	22.9	17.9	32.5	19
25	25.3	19.0	35.8	19

TABLE I. DATA ON WHITE MALE RATS (UNMATED), SHOWING INCREASE IN WEIGHT OF BODY WITH AGE. — *Continued.*

AGE IN DAYS.	BODY-WEIGHT IN GRAMS.			NUMBER OF ANIMALS.
	Average.	*Lowest.*	*Highest.*	
27	27.4	19.8	38.3	19
29	29.5	22.1	39.3	19
31	31.8	25.9	41.2	19
34	34.9	27.4	43.3	19
37	37.8	28.5	48.0	19
40	42.2	30.8	52.2	19
43	46.3	33.7	62.4	19
46	50.5	35.9	66.2	19
49	56.7	38.9	73 9	19
52	62.5	39.8	82.5	19
55	68.5	40.6	87.5	19
58	73.9	45.1	100.1	19
61	81.7	49.0	116.6	19
64	89.1	52.7	129.6	19
67	99.3	57.7	140.2	19
70	106.6	71.2	148.5	19
73	113.8	71.4	152.4	19
76	121.3	89.8	157.5	19
79	128.2	97.0	161.2	19
82	135.0	105.1	165.5	19
85	143.8	117.0	168.5	19
88	148.4	124.5	174.0	19
92	152.3	124.0	179.6	19
97	160.0	124.0	180.7	19
102	168.8	120.0	192.2	19
107	177.6	120.0	206.0	19
112	183.8	125.0	215.6	19
117	191.4	130.0	223.0	19
124	197.3	123.0	238.2	19
131	202.5	132.4	249.2	19
138	209.7	145.6	248.4	19
143	218.3	155.5	259.4	19
150	225.4	162.4	268.2	19
157	227.0	162.4	271.4	19
164	231.4	159.0	271.8	17
171	235.8	165.2	289.0	17
178	239.4	167.9	291.2	17
185	239.8	176.0	294.0	15
216	252.9	190.5	294.5	10
256	265.4	190.5	310.0	10
365	279.0	203.6	320.0	6
730	308.5	285.0	375.6	6

TABLE II. DATA ON WHITE FEMALE RATS (UNMATED AND MATED),[1] SHOWING INCREASE IN WEIGHT OF BODY WITH AGE.

AGE IN DAYS (Gestation 21 Days).	BODY-WEIGHT IN GRAMS.						NUMBER OF ANIMALS.
	Average.		*Lowest.*		*Highest.*		
Birth	5.2		4.2		6.2		17
1	5.5		4.5		6.1		11
2	5.7		4.8		6.3		7
3	6.2		5.6		6.5		9
4	6.5		5.6		7.0		10
5	7.7		7.0		9.0		9
6	8.5		7.1		11.0		11
7	8.7		7.5		11.8		8
8	10.6		7.1		13.1		13
9	11.1		9.4		12.6		9
10	12.1		9.1		14.4		6
11	12.8		12.1		13.6		2
12	15.1		13.6		17.7		5
13	15.1		14.7		16.0		5
14	15.6		13.5		18.1		5
15	17.7		13.1		23.2		17
17	19.2		15.1		24.5		17
19	20.6		16.9		27.0		17
21	22.6		16.1		30.1		17
23	24.9		17.3		33.3		17
25	27.4		20.8		36.0		17
27	30.0		23.9		38.5		17
29	31.4		24.0		39.0		17
31	32.9		26.3		42.8		17
34	35.7		26.4		44.1		17
37	39.5		29.8		47.4		17
40	43.7		30.6		52.4		17
43	47.9		35.0		60.7		17
46	52.0		41.4		63.0		16
49	57.7		42.0		69.2		16
52	62.9		41.7		74.8		16
55	68.4		49.8		80.7		13
58	74.6		53.6		86.6		13
61	78.4		56.2		96.7		13
64	85.8		57.5		106.8		12
67	96.0		71.2		114.1		12
70	99.8		79.0		122.6		11
73	105.6		80.2		126.5		11
76	110.4		89.6		131.6		11
79	118.8		97.7		136.0		11
		Mated.		*Mated.*		*Mated.*	
82	124.7	—	101.0	—	139.2	—	11
85	131.5	—	105.0	—	143.2	—	11
88	136.0	—	115.6	—	157.4	—	11
92	139.6	139.8	118.7	118.9	161.4	161.6	11
97	145.9	146.3	119.6	120.0	174.5	175.0	11
102	152.4	153.1	124.6	125.2	185.7	186.5	11
107	154.9	155.8	129.6	130.3	191.4	192.5	11
112	160.2	161.4	138.5	139.5	193.6	195.0	11
117	166.5	168.0	142.5	143.8	199.0	200.8	11
124	170.7	172.6	146.4	148.0	206.7	209.0	11
131	178.6	181.0	151.2	153.0	214.7	217.5	11
138	182.2	185.0	151.0	153.3	210.2	213.4	11

[1] Under "Mated" are given the *estimated* body-weights for rats allowed to breed.

TABLE II. DATA ON WHITE FEMALE RATS (UNMATED AND MATED), SHOWING INCREASE IN WEIGHT OF BODY WITH AGE. — *Continued.*

AGE IN DAYS.	BODY-WEIGHT IN GRAMS.						NUMBER OF ANIMALS.
	Average.		*Lowest.*		*Highest.*		
		Mated.		*Mated.*		*Mated.*	
143	183.4	186.6	154.0	156.7	219.4	223.4	11
150	184.6	188.2	153.7	156.7	220.7	225.0	11
157	184.0	188.0	154.9	158.2	217.6	222.4	11
164	185.1	189.5	154.0	157.6	215.0	220.1	11
171	187.4	192.2	154.0	158.0	210.0	215.4	11
178	191.7	197.0	153.0	157.2	215.0	221.0	11
185	194.2	200.0	152.0	156.6	215.0	221.4	11
192	195.9	202.2	155.0	160.0	217.0	224.0	11
365		226 4		171.4		280.0	7

TABLE III. CALCULATED GROWTH OF A RAT IN WEIGHT BEFORE BIRTH, BASED ON THE OBSERVATIONS OF FEHLING ON THE RABBIT FŒTUS (SEXES NOT DISTINGUISHED).

RABBIT.				RAT.	
Observed Growth of Fœtus.				Calculated Growth of Fœtus.	
Age in Days.	*Period.*	*Weight of Fœtus in Grams.*	*Ratio.*	*Age in Days.*	*Weight of Fœtus in Grams.*
12	5	.619	1	9	.087
15	6	6.167	10	11	.870
18	7	11.734	20	13	1.750
21	8	18.650	30	15	2.610
24	9	28.908	47	17	4.100
27	10	33.670	54	19	4.700
30–31	Term.	38.350	62	21	5.400

DATA ON MAN.

The figures for man have been taken for the most part from ROBERTS'S tables (1878). These are reproduced in Table IV; the weights, originally given in pounds avoirdupois, being changed to kilos, 2.2 pounds being taken as equal to 1 kilo. Unfortunately, the records for the male are incomplete at one year and at two years, no record being made in the first instance, and in the second that given being an average of only two observations. It is therefore necessary to supplement the curve at this point. The emendations which have been made are entered in parentheses at the right of Roberts's observa-

tions, and are based on figures published by CAMERER (1893). As will be seen, this emendation, based on three individuals weighed at two periods, makes the weight at one year 9.9 kilos, and at two years 12.8 kilos. The form of the curve given by these numbers corresponds very closely with that based on the record for the female (Roberts), and also with that obtained by Dr. MISHIMA (1904), in his careful study of Japanese children of both sexes during the first fifteen years of life.

It should, however, be further stated that in Roberts's records, the children at birth were weighed without clothing, while the records for all the other ages give a weight in which indoor clothing is included. This, of course, modifies the form of the curve between birth and the end of the first year; but after that point its influence on the shape of the curve can for our purpose

TABLE IV. DATA ON MAN (MALES AND FEMALES), SHOWING INCREASE IN WEIGHT OF BODY WITH AGE.[1]

AGE IN YEARS (Gestation 285 Days).	BODY-WEIGHT IN KILOS.			
	No. of Cases.	*Males.*	*Females.*	*No. of Cases.*
Birth	451	3.2	3.1	466
1	— (3)	— (9.9)	9.1	8
2	2 (3)	14.5 (12.8)	11.5	9
3	41	15.4	14.4	30
4	102	16.9	16.4	97
5	193	18.1	17.8	160
6	224	20.1	19.0	178
7	246	22.6	21.6	148
8	820	24.9	23.7	330
9	1425	27.4	25.3	535
10	1464	30.6	28.2	495
11	1599	32.6	31.0	456
12	1786	34.9	34.7	419
13	2443	37.6	39.7	209
14	2952	41.7	44.0	229
15	3118	46.6	48.3	187
16	2235	53.9	51.4	128
17	2496	59.3	52.5	74
18	2150	62.2	55.1	64
19	1438	63.4	56.4	97
20	851	64.9	56.1	128
21	738	65.7	55.5	59
22	542	67.0	56.1	53
23	551	67.0	56.4	29

[1] From Roberts's tables (1878), except males at one and two years, the data for which are interpolated in parentheses from Camerer's records, weight without clothing. So far as Roberts's records are concerned, the weight, except that at birth, includes the weight of indoor clothing.

be neglected. In the data taken from Camerer for the first and second year of the males, the weights are without clothing, and hence at those periods the weight of the male unclothed is compared with that of the female clothed. The amount to be added to the male, in order to adjust the difference, is probably from 6.5 per cent. to 7.2 per cent. of the true body-weight (see BOWDITCH [1877]), or .65 to .72 kilos at one year, and .83 to .92 kilos at two years. In the absence of exact data, we have not attempted any modifications of the figures as given by Camerer and reprinted in Table IV, but have entered on the chart the values without correction for clothing.

It is also important to enter for man, as has been done for the rat, the curve of growth from conception to birth. For this purpose, we have used the data furnished by FEHLING (Table V).

TABLE V. DATA ON MAN, SHOWING GROWTH OF HUMAN FŒTUS AT THE BEGINNING OF THE PERIODS INDICATED (SEXES NOT DISTINGUISHED).

Age in Lunar Months.	*Weight in Grams.*
Second month	1
Third month	7
Fourth month	20
Fifth month	120
Sixth month	285
Seventh month	635
Eighth month	1220
Ninth month	1700
Tenth month	2240
Term	3250

The portion of the curve for man in Plates II and III, between conception and birth, is based on the figures in the foregoing table.

CONSTRUCTION OF CHART IN PLATE II.

As previously mentioned, the base-line on which the ordinates for these growth-curves are erected has been so adjusted that one year of rat life equals thirty years of human life, the smaller intervals of time being given their proportionate values.

In entering the records to form the curves, it was found that

the best comparison could be obtained if they were related so that, on the axis of ordinates, 1 mm. equalled 1 gram of rat body-weight, and 1 mm. equalled 250 grams of human body-weight.

This was the scale of the original drawing, which has been reduced for reproduction. The data used for the curves are those in

Table III, for the fœtal rat.
" I, for the male rats.
" II, for the female rats.[1]
" V, for man before birth.
" IV, for man after birth.

In the case of man, the weights are taken from Table V, the values given by Camerer for the male at one and two years being employed.

As Minot (1891) has indicated, it is important to begin the growth-records of this sort at the fixed point of conception. The period of gestation in man is here taken to be two hundred and eighty-five days, and in the rat twenty-one days. As the general relation of the two life-spans is as 1 to 30, we might expect that the period of gestation for man would be thirty times as long as that for the rat. As a matter of fact, it is only about fifteen times as long; so that, in the curves as they are drawn, the time of birth for man comes relatively earlier than for the rat.

When we take into consideration the great immaturity of the rat and its relatively long period of gestation, there seems to be but one conclusion possible; namely, that in the fœtal rat as compared with man the growth-processes are decidedly feeble.

PHASES OF THE GROWTH CURVE. — In all cases where records have been made, — i.e., man, rabbit, and the guinea-pig (Minot), — fœtal growth is represented by a curve which rises first slowly, then rapidly; the more rapid rise appearing during the second half of fœtal life.

[1] The calculated weights for breeding females between the ninety-second and one hundred and ninety-second day in Table II were those used in making the curve.

Leaving aside for a moment the interpretation of this curve in terms of the rate of growth, it is to be observed that there is in man a period of rapid rise, beginning at the middle of gestation, and continuing during the first year of extra-uterine life; while in the rat (the curve for which is based on the rabbit) the same event is entirely completed at the time of birth. Minot's observations on the fœtal guinea-pig also show this phenomenon of a rapidly rising curve at this time. Such being the case, it is possible to recognize between conception and maturity five phases in the growth-curve,—phases which are characterized by variations in the rise of the curve.

To facilitate the identifications of these several phases of growth in the curves which are here given, the following table is presented to show how they are marked off.

TABLE VI. PHASES OF GROWTH (MALES ONLY).

	TREND OF CURVE.	MAN (MALES).	RAT (MALES).
Phase 1.		First 140 days of gestation.	First 9 days of gestation.
Phase 2.	More rapid rise.	From 141st day of gestation to end of first year.	From the 10th to the 17th day of gestation.
Phase 3.		From beginning of 2d year to the 5th year.	From the 18th day of gestation to the 2d to the 7th day after birth.
Phase 4.	More rapid rise.	From beginning of 6th year to end of the 16th.	From the 3d to the 8th up to the 70th day.
Phase 5.		From the 16th year to maturity.	From the 71st day to maturity.

The limits of these phases are so modified by sex, that certainly Phases 5 and 4 in the female curve come earlier, and the same is probably true of Phase 3. Concerning Phases 2 and 1, we cannot at the moment speak, as the form of the curve given for the rat is merely inferred from observations on the rabbit, and the sexes are treated together.

COMPARISON OF CURVES FOR THE TWO SEXES.—In comparing the curves for the two sexes in man, we find the well-known differences whereby in Phases 2 and 3 the female shows a smaller body-weight than the male. Somewhat beyond the

middle of Phase 4 the female grows more rapidly, and is for the time heavier. It should be remembered in this connection that the length of time during which the weight of the female remains greater than that of the male is much longer in a curve of the sort we are using than it usually is in the case of selected pairs. At the end of the fourth phase the female grows less rapidly, the curves recross, and the relations characteristic of maturity are attained.

On examining the corresponding curves for the rats (Plate II) we see the same relations repeating themselves in the same phases of the growth-curve.

As has been stated before, in the case of the rat the second phase is completed before birth; and the third phase then becomes evident, and continues for a few days after that event. In our present records it will be seen that the more active growth of the female begins as early as the seventh day after birth, and that in this particular set of observations the two curves cross about the fifteenth day. They remain crossed until the fifty-fifth day, when, after a few days of fluctuation, they recross and separate permanently.

In the rat, therefore, the curve for weight of the female is related to that for the male in the same way as are the corresponding curves in the case of man. In this instance, then, we have an animal widely removed from man in the zoölogical scale, belonging to an order palæontologically ancient, and exhibiting phylogenetically but a slight tendency to variation, which shows a series of growth-relations similar to those observed in man.

In a certain sense the purpose of this presentation is accomplished when we have shown in what relation the growth-curves of these two animals stand to one another. One is tempted, however, to go a step further, and call attention to the possibility that mammals as a class may grow in a like manner. This point has already been raised by Minot on the basis of his own observations of the guinea-pig, which, if I interpret them correctly, show a curve of growth for that animal which gives a long fifth phase preceded by a comparatively short fourth and still shorter third phase.

During the last of the third and beginning of the fourth, for about the first twenty-eight days of post-natal life, the female is slightly heavier than the male. This was the first instance where more vigorous growth in the female in lower mammals was noted and described.

Though not of an equal value with those just presented, there are some other records in the literature which seem to confirm the existence of this general relation.

CORNEVIN has tables (1892) showing the weight of growing cattle. These tables do not give all the information necessary; but, if curves for the growth of the two sexes in body-weight be plotted from the data as given on p. 481 of his paper, it appears that between the first and fourth months of post-natal life the female is heavier than the male.

Since in this species puberty occurs during the first year, this relation may express the pre-pubertal acceleration in the female. As the matter stands, however, the evidence is not very conclusive.

Ménard followed the growth of eight giraffes, taking the height at the withers. His measurements show a pre-pubertal superiority of the females at two years, puberty in this animal coming between the ages of three and four years. After puberty, the males have the greater height. Ménard remarks that the general size of the animals corresponded with the differences in the measurements which he has given, so that we can infer that they would probably have differed in weight in the same sense.

In his article on growth, in HERMANN'S "Handbuch der Physiologie," Vol. VI, 2, p. 262, the author, HENSEN (1881), gives a table showing a relatively greater rate of growth of young female guinea-pigs during the first fifty-one days of life. If, however, I interpret his figures correctly, the *absolute* weight of the females at this time was regularly less than that of the corresponding males, despite the more rapid rate of growth which the females exhibited.

In this connection it may be stated that the literature does not show any records by which opposite relation (i.e., more rapid growth of male) is demonstrated, — a fact which adds

weight to the foregoing interpretation of these imperfect data. A word of caution is needed, however, against linking too closely the period of more rapid growth of the female with puberty. It so happens that in man the two events are nearly related; but, taking the other cases that have been well worked out, we find in the guinea-pig that at twenty-eight days the female is already growing more slowly than the male, though sexual maturity does not occur until the hundred and twentieth day; while in the rat, puberty is not attained until the sixtieth day at the earliest, at which time the female is growing less rapidly than the male, the onset of the rapid growth of the female having appeared about the eighth day of life. Our information in the case of cattle and the giraffe is not complete enough to warrant comments. The relation of these two events is therefore less close than a study of the data on man alone would suggest.

To prevent any misunderstanding concerning the significance to be attached to the direction of the curves here used as indications of the several phases, it is necessary to add one or two words on their general interpretation. Where the record for the change of weight appears as a straight line, we know that this indicates equal absolute increments in equal times. If, however, at any point in its course, the curve bends towards the horizontal, it indicates that for that period the absolute increment is less than for the preceding, and if it bends towards the vertical, that it is greater. It appears, however, as has been pointed out by Minot, that the rate of growth, as measured by the percentage increase from interval to interval, diminishes, *in all cases* observed, very rapidly and with but slight fluctuations from the earliest moment at which we can measure the growth-process up to the end of the growing period. The curve necessary to represent an *equal rate of growth* would be an exponential curve approximating the vertical with greater or less rapidity, according to the value of the rate.

From this it follows, that, in the cases before us, we are always dealing with a diminishing rate, and that the variations occur merely in the rapidity with which this diminution takes place. Hence in the several phases to which we have drawn

attention, the parts of the curve showing rapid rise or slow rise, while they indicate variations in the increment of the absolute weight of the animal, at the same time indicate changes which represent in general a rapid though slightly varying diminution in the rate of growth.

Cause of the Phases.

Before leaving the study of these records, it seems desirable to consider for a moment the explanation of the several phases.

We recognize, in the first place, that the growth with which we have to deal is dependent mainly upon cell-multiplication and cell-enlargement; further, that after several classes of cells arise within the organism, it is probable that we have to deal with modifications in the growth of one class due to the activities of others. If we survey the span of life from the beginning to the end, we find that in the first and second phases, principally, cell-division is very active, while in all the later phases cell-enlargement is the main cause of the increase in total size. Moreover, the last process is influenced by the number of cells which in each species is destined to undergo enlargement.

Since, in the rat, birth occurs during the third phase, cell-division as a factor in growth would appear to be comparatively insignificant between birth and maturity, cell-enlargement being the chief cause for the changes taking place.

A priori, we might expect that this process of enlargement would give us a simple steadily rising curve which rather rapidly turned and flattened as maturity was approached. Since the curve departs clearly from this form, having after birth three well-marked phases, in the first and last of which it rises slowly, separated by one (fourth phase) in which it rises more rapidly, it seems highly probable that the enlargement of the body as a whole is a resultant of the complex influences represented by the interaction of several systems; but into this division of the topic it is not our purpose at present to go. We note, however, that birth in man is relatively an early event, while puberty comes later—something more than halfway from birth to maturity; the relative interval between birth and puberty being nearly three times as long in man as in the rat.

As a result of this study, we conclude that man and the rat attain their adult weight after having passed through a series of phases similar for both animals; and that, moreover, in both of them the increase in body-weight in the two sexes is related in the same way.

Details on the Period of More Rapid Growth of the Female Rat.

Before closing this paper it will be desirable to give a little more detail bearing on that part of the rat curve in which the growth in the female is more rapid than in the male.

TABLE VII. Data on the White Rat, being Dr. Dunn's Records of the Average Weights of the Individuals composing Different Litters from Birth to 14 Days, arranged by Litters.[1]

Age in Days.	Weight of Body in Grams.			
	No. in Each Litter.	*Males.*	*No. in Each Litter.*	*Females.*
Birth	4	5.77	4	5.26
1 day	3	6.01	2	5.69
" "	4	5.96	4	5.86
2 days	2	6.06	3	6.02
3 "	5	6.26	5	6.19
3 "	3	6.35	4	6.13
4 "	6	7.06	6	6.52
4 "	4	6.79	4	6.45
5 "	3	9.56	3	8.43
5 "	2	7.88	2	7.46
5 "	4	7.57	4	7.16
6 "	4	8.10	3	7.69
6 "	3	11.99	3	10.54
6 "	4	7.91	5	7.77
7 "	3	7.49	2	7.88
7 "	3	8.19	2	7.61
7 "	2	7.92	2	8.60
7 "	3	12.59	2	10.77
8 "	4	10.35	3	10.76
8 "	4	12.43	3	11.80
8 "	2	11.30	3	12.58
8 "	4	7.84	4	7.40
9 "	3	12.66	2	11.99
9 "	3	12.22	3	12.23
9 "	4	9.70	4	9.76
10 "	3	13.27	2	13.87
10 "	3	11.22	4	10.28
11 "	4	13.25	2	12.84
12 "	3	16.53	3	15.89
12 "	3	13.06	2	14.22
13 "	5	15.29	5	15.10
14 "	4	14.64	3	14.58
14 "	2	16.27	2	17.12

[1] Except for the ages, birth, one day, and two days, the average from the observations given above are used in Tables I and II.

If we analyze Dr. Watson's records, we find that there were seven out of ten litters in which both sexes were carried through the *entire* period of observation. In these seven litters, six of them show the female to be heavier at some time between the fifteenth and seventy-fifth day of life. In the seventh litter, the weight of the female, although always less, was closest to that of the male between the fortieth and fiftieth days. This seems to be ample evidence for the more vigorous growth of the female during the third and beginning of the fourth phases. Dr. Watson's observations, however, begin with the fifteenth day of life, at which time the female is already the heavier; and it was for Dr. Dunn to determine from her observations how the two sexes were related during the first fourteen days. The accompanying table (VII) shows the average weight of the separate litters, from which the weight of the rat during the first fourteen days has been determined. Where the average for the female is heavier for a given litter, the figures are printed in heavy-faced type.

For the ages birth, one day, and two days, Dr. Dunn's records have been supplemented by adding from the laboratory archives the following, the totals being also found in Tables I and II.

Age.	Number of Animals.					
	Males.			*Females.*		
	Dunn.	*Archives.*	*Total.*	*Dunn.*	*Archives.*	*Total.*
Birth	4	36	40	4	13	17
1 day	3	23	26	2	9	11
2 days	4	6	10	4	3	7

It appears that during the first six days of life, the average for the females is always less than that for the males. Beginning with the seventh day, and between this and the fourteenth day, nine out of the nineteen litters show an average for the female greater than that for the male. As reference to the table will show, these instances occur on the seventh, eighth, ninth, tenth, twelfth, and fourteenth days, but in the grand averages these relations are overbalanced, except on the eighth, twelfth, and fourteenth days. We infer from this that the

period of more vigorous growth in the female begins about the seventh day of post-natal life.

Of course, from the biological standpoint, it makes no difference whether the curves of weight cross or not; the important change is the more rapid growth of the female during this early period. To better illustrate these events during the first seventy days of life, the first part of the curve (Plate II) has been enlarged to four times the scale on which it is there drawn, and is shown in Plate III. As to the time of recrossing, or the beginning of the slower growth of the female, which our curves show at the beginning of the fifth phase, we have another series of observations by Dr. Dunn on five litters.

In all these five litters there was a time during the fourth phase when the female was growing more rapidly than the male. The change in the rate of growth by which the female grows more slowly again may occur any time between the twentieth and sixtieth days, while on the average the growth in the female is found to be most rapid, as compared with the male, between the twenty-fifth and thirtieth day of life. After this, of course, begins the relatively slower growth of the female, which sooner or later leads to the relations of weight characteristic of maturity. There is, then, no question that the relations expressed by Dr. Watson's records are entirely correct, in that they show a relatively more vigorous growth of the female during the fourth phase, with the usual result of making the females absolutely heavier at this time.

Conclusions.

I. Applying to both Rat and Man.

1. The curves recording the increase in the body-weight of man and of the white rat between conception and maturity exhibit similar phases, five in number.

2. The growth of the female, in relation to that of the male, is similar in both forms, as are also the relative weights of the two sexes at maturity.

II. Applying to the Rat only.

3. In the rat, as compared with man, the period of gestation is a larger fraction of the span of life, and puberty comes rela-

tively earlier, and longer before the mature body-weight is attained.

4. The age of puberty in the rat (60–70 days) is separated from the onset of more rapid growth in the female by a relatively long interval. The two events are therefore not necessarily closely associated.

5. In the rat, increase in body-weight during the last phase is continued for a relatively longer time than in man.

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EXPLANATION.

In the original drawing, 1 mm. = 1 gram of body-weight in the rat, laid off on the ordinate to the left; and 1 mm. = 250 grams of body-weight in man, laid off on the ordinate to the right in kilograms.

On the base-line, 1 mm. = 1 day of rat life, 12.15 mm. = 1 year of human life, and the zero-point is taken at the time of birth. To the left of the zero-point, 21 mm. are laid off, corresponding to the 21 days of gestation for the rat; and 9.4 mm. are laid off, corresponding to the 285 days of gestation for man.

The point of conception (*C*) coincides for the two curves; but as gestation in man is relatively only half as long as in the rat, and as the ages are in both cases counted from birth, the two curves are somewhat displaced, so that the 30th year of human life falls a little to the left of the 365th day of rat life.

The lines showing body-weights are heavier for man than for the rat; and in each case the curve for the male is indicated by the solid line, and that for the female by the broken one. For the records before birth no distinction for sex is made, and the solid line is used.

Where the curves for the two sexes run close together, the distances have been exaggerated in some instances in order to keep the lines distinct.

Chart reduced to one-third of the original dimensions.

A NEW LABORATORY PROJECTION APPARATUS

BY

M. J. GREENMAN

The Wistar Institute of Anatomy, Philadelphia, Penna.

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The Wistar Institute of Anatomy, Philadelphia.

WITH 10 FIGURES.

The projection apparatus was designed to meet the requirements of the anatomical laboratory of the Wistar Institute where, in nearly every research, photographic processes, outline drawings from the projected objects or Born's method of reconstructing microscopic objects are employed. It is essentially a fixed apparatus and not designed for lecture

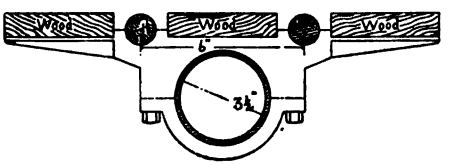

FIG. 1.

purposes. Its parts are all heavy to avoid vibration and that they may remain in place without fastenings. The base or optical bench differs from other forms of projection apparatus in consisting of one piece. Other differences are found in the mechanical stage, in the cooling cells, in the focusing apparatus, in the lantern, and in some other minor points. The camera presents a number of new features, but as it is not yet completed no further mention will be made of it at this time, except to say that it is to be applied to the same optical bench and the same lantern, condensers, mechanical stage, and focusing device are to be used.

The apparatus is a result of a series of experiments with crudely constructed apparatus to ascertain the exact requirements. I am indebted to Dr. H. D. Senior and Dr. G. L. Streeter for their assistance in developing the plans and to Mr. S. Noble, the Institute's mechanician, for the skillful mechanical work.

The Optical Bench consists of an iron frame approximately ten feet long. It is made up of a central steel tube 3½" in diameter bearing

eight saddles or transverse castings placed equidistant upon this tube. The tube furnishes rigid support for the transverse bars which in turn bear two parallel round steel shafts or ways each 1⅛″ in diameter and 10′ long and set 6″ from centre to centre. Between the two steel ways and on both outer sides are strips of wood 4″ x ⅞″ secured to the saddles forming a flat table surface. The central strip bears a T slot which may be used to secure apparatus to the bench. See Fig. 1.

Accurate alignment of all the optical parts is secured by turning and grinding to a perfectly true cylinder the supporting 3½″ tube; milling all the saddles on a jig so that they are exactly alike and using turned and ground shafting for the ways.

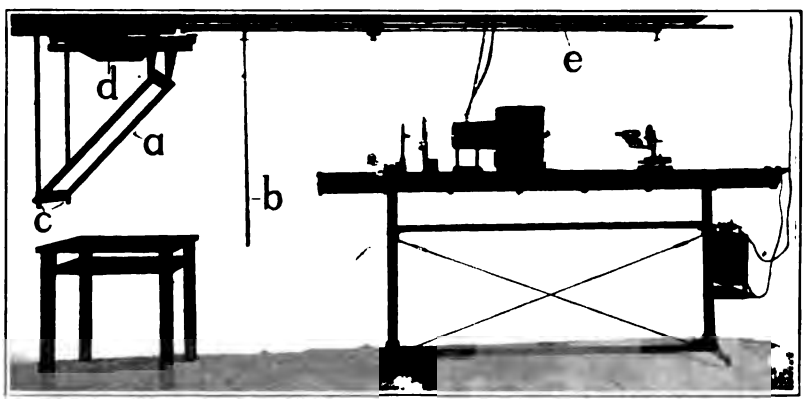

FIG. 2.

The optical bench is borne upon two upright tubes or standards 2¼″ in diameter mounted in cast iron feet and of the proper length to bring the top of the bench 48″ from the floor. This frame is made rigid by a 1½″ channel from foot to foot and two diagonal tie rods with turnbuckles. Just beneath the optical bench is a shelf 10″ wide. Upon a bracket on one standard is mounted the Rheostat (10 to 25 amperes). Upon the two steel ways the lantern, condensing system, mechanical stage, microscope, and other accessories are movable from end to end of the bench.

At the proper distance from this apparatus is placed the mirror and drawing table. The mirror 24″ x 36″ (Fig. 2 a) is suspended at an angle of 45° from a wooden framework by strips of steel, one pair of which has binding screws and slots at the lower ends (c) in order that

the miror may be accurately adjusted at 45°. The wooden framework carrying the mirror is 36″ x 33″; the central cross bar (d) is blocked down at each end in order to pass beneath the apparatus secured to the ceiling. This frame is suspended from the ceiling of the projection room by means of a steel track such as is used for sliding doors in house construction. This makes it possible to move the mirror nearer to or farther from the projection apparatus according to the magnification desired. The drawing table is of ordinary construction with a shelf just beneath the top to carry paper or wax plates. Suspended by a universal joint

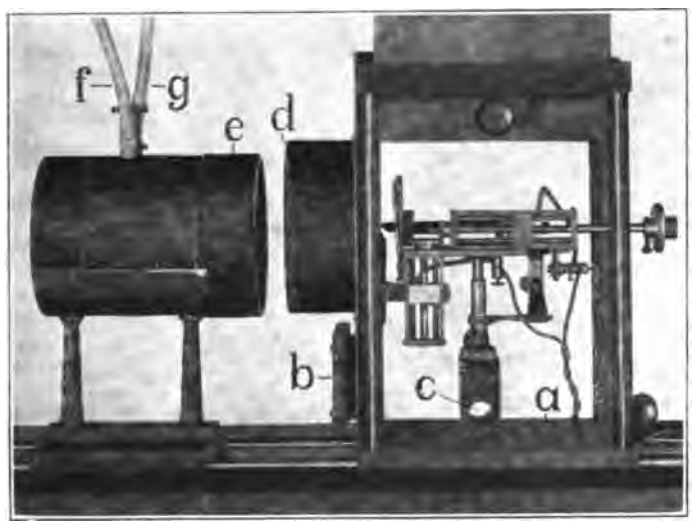

FIG. 3.

conveniently near the drawing table is the focusing rod (b) which is also movable from end to end of the projection room. Secured to the ceiling by brackets are two parallel steel rods (e) $\frac{1}{2}$″ in diameter and $2\frac{1}{2}$″ apart. These rods extend from end to end of the projection room directly above the apparatus. They carry the pulleys of the focusing apparatus and make it possible to have the microscope at any point of the optical bench and focus it while working at any other point in the room. Projecting from the ceiling, at convenient intervals over the optical bench are three pairs of water supply ($\frac{3}{8}$″) and waste pipes ($\frac{1}{2}$″) (not shown in the figure) to furnish water circulation for the cooling cell.

Each piece of apparatus to be used on the bench has a cast iron base,

the bottom of which has a V-shaped groove on one side and a flat surface on the other side. The V-groove fits over one way or shaft of the optical bench and keeps the apparatus in line while the flat surface rests on the other shaft giving the necessary support.

The lantern consists of a cast iron base (Fig. 3a) $10\frac{3}{4}''$ x 8″. The corner supports are $\frac{1}{8}''$ turned rods into which grooves are sawn 1/16″ wide by 3/16″ deep. In these grooves the sides of the lantern slide. Any side may be drawn out as shown in Fig. 3. The top of the lantern is open, yet made light proof by a series of Z-shaped sheets of metal. Through the base at (b) and at (c) are air inlets. At (d) is a double tube or light lock secured to the front of the lantern into which an extension of the condensing system (e) fits loosely so that the distance from the arc to the condensing lens may be varied without allowing light to escape. The lamp which we use is a Thompson Hand Feed Lamp. The screws for adjusting the carbons extend through the rear of the lantern

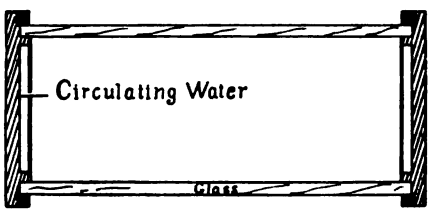

FIG. 4.

and by means of a strip of steel held in grooves in the rear plate render the adjustment of the lamp easy and without the escape of light. The electric wires enter the lantern through two small holes extending 3″ into the base and then turning upward into the lantern. The optic axis is fixed at 9″ from the top of the ways of the bench.

The condensing system consists of two 6″ plano-convex lenses in extra heavy brass mountings with a cooling cell fitted snugly between to absorb the maximum amount of heat. The cooling cell differs from other forms known to me in having a hollow or jacketed wall through which cold water, from any source, keeps the temperature down. The water does not enter the cell itself. The water flows into one opening (f) passes almost entirely around the cell and out another opening (g). The construction is shown by the section Fig. 4. Water connections are made with the before mentioned outlets in the ceiling of the projection room by means of rubber or flexible metal tubes with screw fittings. This device keeps the lens mountings cool and renders the rays practically free from heat. In

cases where extra light is needed or where objects must be kept cold a second cell is attached to the stage. For this improvement I am indebted to Professor Simon H. Gage, of Cornell University, who discovered that when a microscope slide under an intense light, bearing heat rays, was placed upon a cold surface the heat was more rapidly conducted out of the slide than it was absorbed from the rays by merely passing through a cold fluid. The glass surface of the cooling cell is therefore the stage proper. Behind the cell is a brass plate carrying an iris dia-

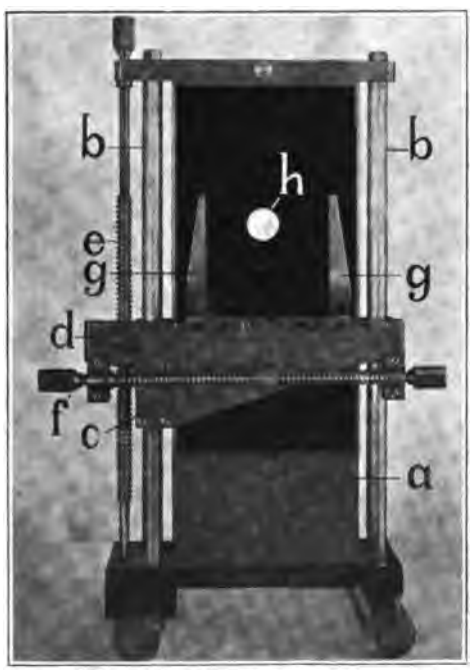

FIG. 5.

phragm. When the cooling cell is not needed the brass plate is brought forward to serve as the stage proper. The cooling cell is 4½″ wide, 9″ high, and 1½″ thick.

The mechanical stage differs from others in its capacity. An ordinary 1″ x 3″ or a 7½ x 5½″ slide are equally easily manipulated. The screws, especially made for the apparatus, give a moderately rapid movement of the carrier in either direction. Referring to Fig. 5, (a) is a heavy cast block to give stability without the necessity of fastening it to the bench.

Two $\frac{5}{8}$" steel rods (b b) carry the movable parts (c-d). The vertical movement is accomplished by the screw (e) while the bar (d) is moved horizontally upon (c) by the screw (f). Two adjustable clips (g g) hold by means of beveled edges the glass slides against the stage. These clips may be moved to accommodate any length of slide up to $7\frac{1}{2}$". At (h) is the iris diaphragm, which is placed on the radiant side of the cooling cell. The device carrying the objective consists of a heavy cast iron base (Fig. 6) (a) carrying a sliding top (b); secured to this sliding top by stud bolt (c) is the objective carrier (d) resting upon three adjusting screws (e e e) The objective carrier consists of a large brass disc to which a bellows or other apparatus may be secured. This disc is bored and threaded for our

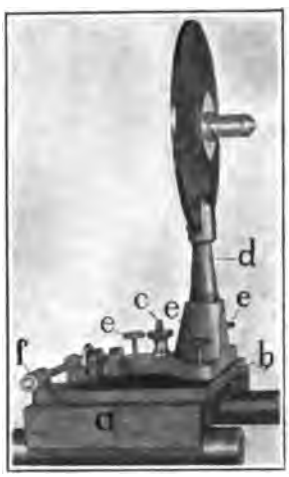

Fig. 6.

Fig. 7.

largest photographic lens and has a series of bushings for other lenses down to the society screw for microscopic objectives.

The fine adjustment is accomplished by the milled head (f) which actuates, through a bevel gear, the sliding plate (b). This simple stand is used for all low powers. When high power objectives are to be used the compound microscope is substituted and mounted on an adjustable table shown in Fig. 7. This table consists of a base (a) through which a 5" hole admits the threaded extension of the table proper (b). The large brass nut (c) projecting slightly on each side is turned to raise or lower the table. The upper portion of the table (d) slides between two beveled guides secured to the lower portion of the table and the

lateral adjustment of the miscroscope is thus accomplished by the milled nut (e) which fits into a groove in the upright (f). The microscope is held in place by the clamp and screw (g) between the guides (h).

The focusing device is shown in position in Fig. 2. The focusing rod (b) hangs from the ceiling and is easily moved from one end of the room to the other, likewise the wheel and jointed arm which actuates the fine adjustment of the microscope may be placed at any point on the optical bench. For the details of the focusing device, Fig. 8 shows a clamp (a) which may be secured to any point along the optical bench by the screw (b); this clamp carries the rod (c) which is adjustable vertically and transversely to the bench by means of the screw (d). At (e) another thumb screw permits the pulley (f) to be adjusted at any

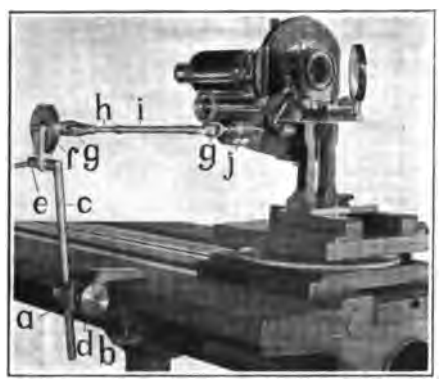

FIG. 8.

angle to the rod (c). At the points (g g) are universal joints, while between them is a shaft (h) carrying a loose sleeve (i). The shaft is grooved on one side and the sleeve carries a key which fits the groove. Thus the arm is freely extensible without clamping screws. The thimble (j) is lined with soft leather and slips over the fine adjustment of the microscope with sufficient grip to turn it. This device is sufficiently flexible in its adjustments and movements as not to bind or jar the microscope while focusing high powers. The pulley (f) is operated by an oiled silk cord or common fishing line coming from the apparatus attached to the ceiling. The details of other parts secured to the ceiling are shown in Fig. 9. Two ½" steel parallel rods 2½" apart (a a) are secured to the ceiling above the apparatus by brackets screwed to the rods at the sides (b b) (brackets not shown) so that the clamps (c c) may pass

without interference. Each pair of clamps (c c) are grooved to fit the rods and clamped together by the screw (j); screwed to the lower halves of these clamps are the pulleys. Pulleys (f) and (g) are idlers, one is placed at each end of the projection room. They are to keep the cord taut over the other pulleys. The axis of pulley (e) extends

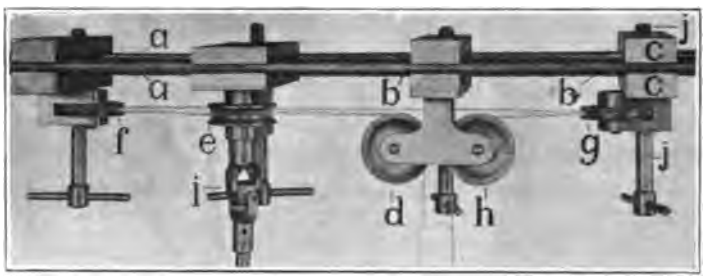

FIG. 9.

downward and carries a universal joint (i) from which the focusing rod is suspended. The cord from the pulley (f) Fig. 8, attached to the optical bench passes over pulley (d) Fig. 9, then to pulley (e) making one complete turn around this pulley, then around pulley (f) and from this point directly to pulley (g) and from (g) over pulley (h) and down to the optical bench again. From the universal joint (i) hangs a wooden

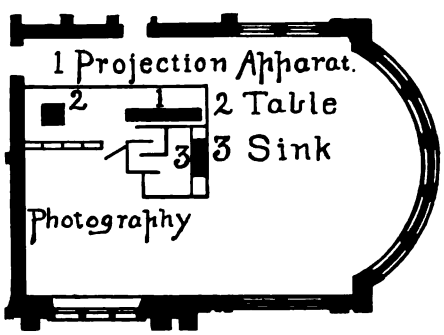

FIG. 10.

rod ¾" in diameter. This rod has been spoken of as the focusing rod and it will readily be seen that a turn of this rod in either direction produces a corresponding movement in the fine adjustment of the microscope. The movement of the focusing apparatus is easy and without

jar, so that while drawing at one end of the projection room, the microscope at the opposite end is easily focused. The same focusing device may be used with the photomicrographic camera.

The projection room together with a dark room of light panel work construction is located in one of the large laboratories so that the investigator may turn from his table to the projection apparatus without leaving the laboratory. The ceiling of the projection room is seven feet high. Good ventilation is maintained by means of a small fan and two 24″ light tight openings in the ceiling and one in the side wall.

Fig. 10 is a floor plan of the laboratory which contains the dark room and projection room and shows the location of the apparatus.

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ON THE ZOÖLOGICAL POSITION OF THE ALBINO RAT

SHINKISHI HATAI, PH.D.

[Reprinted from BIOLOGICAL BULLETIN, Vol. XII., No. 4, March, 1907.]

From the WISTAR INSTITUTE OF ANATOMY AND BIOLOGY, Philadelphia.

ON THE ZOÖLOGICAL POSITION OF THE ALBINO RAT.[1]

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According to Leunis ('83) the black rat (*Mus rattus*) was known in Europe as early as the twelfth century, while the Encyclopedia Britannica (Olfield Thomas, '86) states the appearance of the black rat to be at least as early as the thirteenth century. Although the statements by the different writers as to the appearance of the black rat in Europe do not quite agree, yet it is clear that the arrival of the black rat was much earlier than that of the brown rat (*Mus norvegicus*)[2] which, according to various records, appeared in Europe at about the middle of the eighteenth century, or a little earlier.

Although both species of rats are described as originally natives of Central Asia, yet they are everywhere enemies. By the incessant competition between these two forms, the black rats were almost exterminated, first from Europe, and later from the greater part of North America, and at the end of the eighteenth century, the brown rats were alone found in abundance in these regions.

It is often stated that the white rat at present found in captivity, is the albino of *Mus rattus*. In support of this view there are a number of statements to be found in the older literature (Donndorff, 1792). (No effort has been made to examine the records previous to Linneus).

It is apparently on the basis of these records in the older literature that the current statements in popular natural histories and in encyclopedias are based.

On the other hand, in the zoölogical literature in the nineteenth century, there are numerous statements which refer to the albino rats as a variety of *Mus decumanus*.

[1] From the Wistar Institute of Anatomy and Biology at Philadelphia.

[2] *Mus norvegicus*, Erxleben = *Mus decumanus* Pall. of older Zoölogical Literature. *Norvegicus* has priority, and has come into general use within the last two or three years.

Von Fischer ('69) in a catalogue of the mammals of the St. Petersburg Government, makes the following statement:

"Die Wanderratte, *Mus decumanus* Pall. (russisch Krýssa — Krýssa heist eigentlich *Mus rattus*, diese art ist bekannt unter dem namen Passjúck) kommt ueberall massenhaft vor in allen Farben; schwartz, schmutziggrau bis rostgelb, weissgescheckt und auch ganz weiss. "Die Hausratte, *Mus rattus* L., habe ich nie gefangen, weshalb ich annehmen zu durfen glaube, dass diesse Ratte hier auch nicht vorkommt."

Von Fischer ('74) used a white *Mus norvegicus* in his experiments on the production of hybrids. Later Crampe ('85) also used a white *Mus norvegicus* in experiments of the same nature.

Haacke ('95) and Bateson ('03) studied the crosses between the white *Mus norvegicus* and the common brown rat. None of the authors, however, describe in detail the white forms which they employed.

Despite the general belief to the contrary, there are many reports in recent literature indicating that groups of *Mus rattus* are still to be found in a number of localities, both in Europe and the United States.

In the United States, *Mus rattus* is reported from Texas, Florida and other southern states, and also from Iowa. Rhoads ('03) reports a number of new localities in the States of Pennsylvania and New Jersey. It has been learned through Director Dr. Seitz that in Germany the black rat is present in large numbers in the buildings connected with the zoölogical garden in Frankfurt a/m.

It may be interesting to note that the occurrence of white rats in a wild state has been reported from two localities in Iowa, by students working in the neurological laboratory at the University of Chicago. There are no means of determining, however, whether these were albinos of the black or brown rat. From this review it is evident, therefore, that there are, or have been, at least two forms of albino rats.

Since 1893 a colony of albino rats has been maintained in the neurological laboratory at the University of Chicago, and in 1906 a similar colony was established at the Wistar Institute of Anatomy at Philadelphia.

These colonies have been recruited for the most part from the northern states of the Atlantic seaboard, but some specimens have come from as far south as Missouri. All the rats received from these various localities have appeared to be of the same variety, and have always bred true.

Heretofore, the specific similarity of the albinos and the other forms has been concluded from observation of the external characters only. Wishing more exact information as to the zoölogical relation of the rats composing these colonies, the present investigation was undertaken to determine whether we were dealing with an albino variety of *Mus rattus* or *Mus decumanus*.

Externally, *Mus rattus* is usually distinguished from *Mus norvegicus* by the following specific characters:

Mus rattus is smaller in size. The tail of *Mus rattus* is considerably longer than the body, while in *Mus norvegicus* it is either shorter or only slightly longer than the body, but not relatively as long as that of *Mus rattus*.

The following measurements, though incomplete, serve to indicate this relation:

TABLE SHOWING LENGTH OF BOBY AND OF TAIL.

Observer.	*Mus rattus.*			*Mus norvegicus.*		
	Length Body.	Length Tail.	No. of Obser.	No. of Obser.	Length Tail.	Length Body.
New International Encycl..	21 cm.					27 cm.
Leunis	16 cm.	19 cm.			19 cm.	24 cm.
Hatai				27 males	21 cm.	24 cm.

The general shape of the head (see Fig. 1) of *Mus rattus* is slender, the nose is sharper, and the ear is both wider and longer than in *Mus norvegicus*. It may be worth while to mention that the so-called Alexandrian rat (*Mus alexandrinus*) is said to have external characters similar to those of the black rat (*Mus rattus*) and these two species are only distinguished by their coloring, *Mus alexandrinus* having a brown colored coat.

If we compare the external bodily characters of the albino rat found in our rat colonies, with those of the brown rat, we are surprised by their close similarity. All these characters of the brown rat are also characters of the albino rats composing our

colonies. In other words, the common brown and our albino rats cannot be distinguished from one another by their external characters.

It is nevertheless true that the albino rats which we have examined, are smaller in size than the brown rats in the same localities. In fact, the absolute size of the albino rat is nearly intermediate between *Mus rattus* and *Mus norvegicus*. It is possible

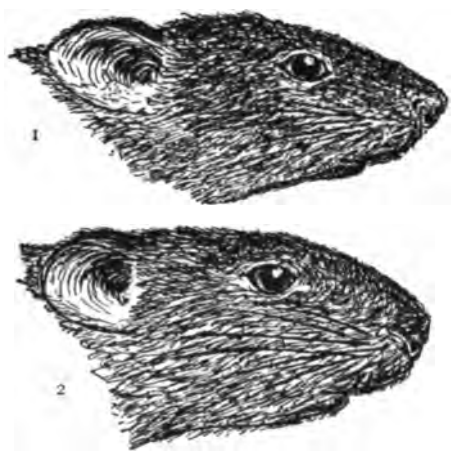
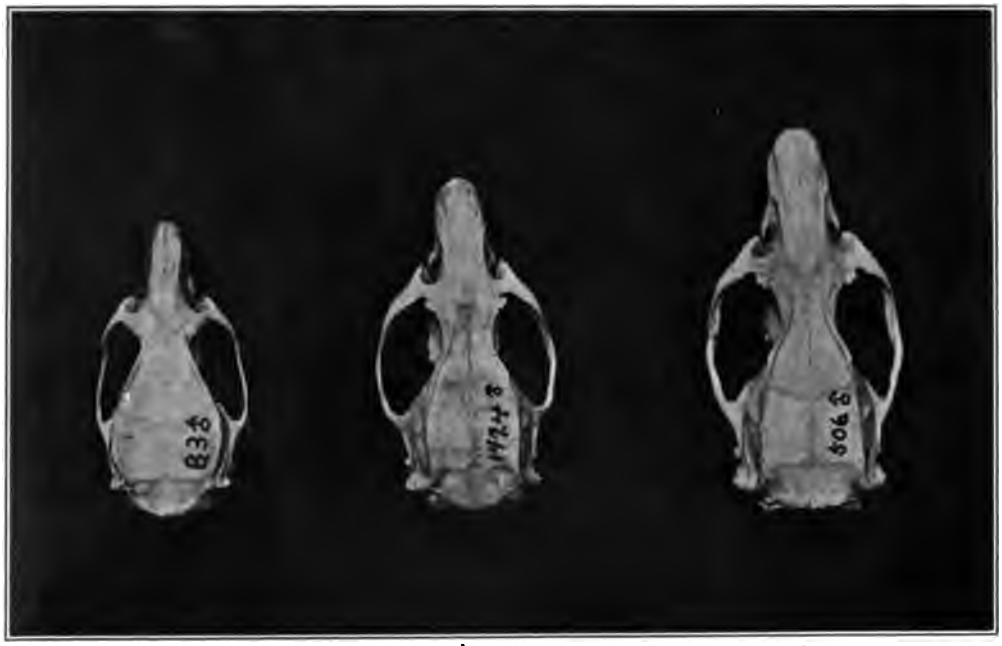


FIG. 1. Copied from "Encyclopedia Britannica," in order to show the shape of the heads of the brown and black rats. 1. *Mus rattus*. 2. *Mus norvegicus*.

that the confinement in which these albinos have been reared, accounts for their smaller size, as the result of lack of exercise and altered conditions of life. It is possible also that we have here a phenomenon similar to that described by Semper ('81) and De Varigny ('94) on snails, where the size of the animals diminished with the size of the vessels in which they were reared.

It was thought that the character of the skull might serve for a more exact distinction of the forms under discussion. We therefore examined and compared the skulls of *Mus rattus*, *Mus norvegicus*, and of the albinos.[1]

[1] In order to make this comparison, it was necessary to examine as many skulls as possible, and I am indebted to Professor J. A. Allen, American Museum of Natural History, at New York, Professor Elliot, Field Columbian Museum at Chicago, Dr. Greenman, The Wistar Institute of Anatomy at Philadelphia, and Professor Merriam, National Museum at Washington, for putting at my disposal various series of skulls, possessed by their several institutions.



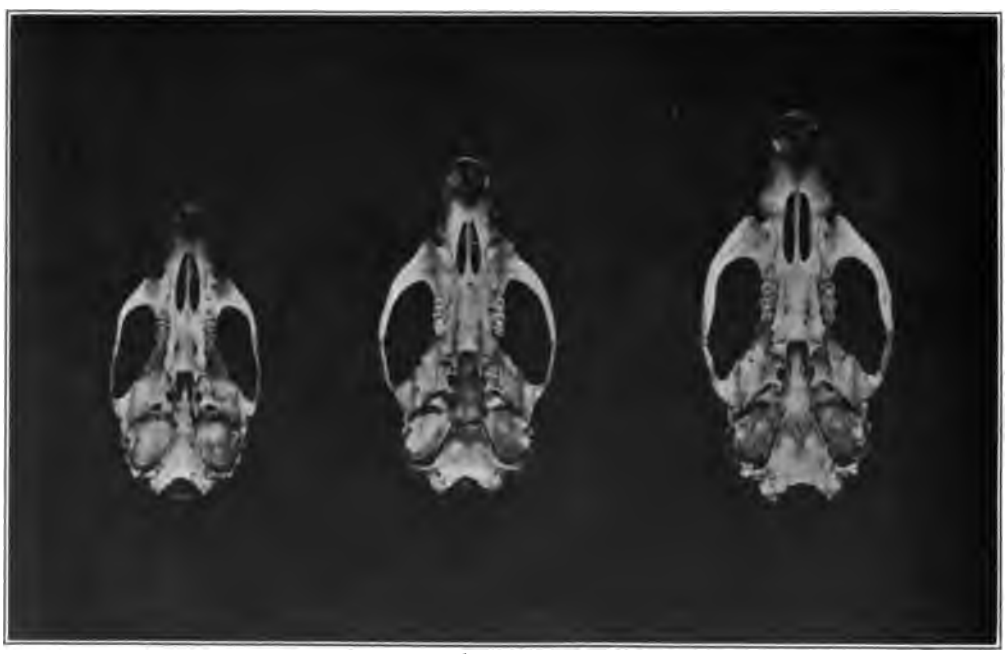


FIG. 2. Shows the skulls of *Mus norvegicus* (*a*), albino rat (*b*) and *Mus rattus* (*c*). The skulls were photographed from two different aspects, in order to show various views of the skulls for a comparison.

The upper row was taken from the dorsal aspect, and the lower from the ventral. The figures are about the natural size.

To illustrate the differences found, both photographs and drawings have been made.

On comparing the skull of *Mus rattus* with the brown rat, the general unlikeness can be seen in Fig. 2.[1] The most noticeable difference is in the shape of the cranium.

When viewed from the dorsal aspect, the cranium of *Mus rattus* is oval in the outline, while that of *Mus norvegicus* is somewhat rectangular. Moreover, the dorsal aspect of the cranium in *Mus rattus* is decidedly convex, while in *Mus norvegicus* it is nearly flat. In *Mus rattus* the os nasale as compared to the entire length of the skull, is relatively shorter than *Mus norvegicus*. In *Mus rattus*, the outline of the os interparietale is somewhat semilunar in shape, while in *Mus decumanus* it is rectangular. In *Mus rattus*, the os parietale is broader as compared with its length, than in *Mus decumanus*. In *Mus rattus*, the foramen magnum is subcircular in outline, while in *Mus norvegicus* it is somewhat rectangular. On the ventral aspect of the skull, the large tympanic bullæ in *Mus rattus* are more conspicuous and eminent than in *Mus norvegicus*.

The junction point of the os basi-sphenoidale and os basi-occipitale is flat in *Mus rattus*, and protrudes in *Mus norvegicus*. The anterior end of the maxilla which forms the lateral wall of the infraorbital fissure, is blunter in *Mus rattus*, than in *Mus norvegicus*. The skulls of our albino rats are very similar in the above characters to those of *Mus norvegicus*, and the description of *Mus norvegicus* may be taken to apply to them.

In connection with the shape of the skulls, the determination of a cranial index has been made. The index used, was that obtained by dividing maximum width of the cranium by the length of the fronto-occipital line. (See Fig. 3.) On account of the small number of specimens measured, the accompanying table is to be considered as merely preliminary, but as it stands it shows a similarity in this index between *Mus norvegicus* and the albino rats, and a difference between these two forms and *Mus rattus*. The cranial index will be made the object of a more extended investigation.

[1] Care has been taken to use only the skulls of fully matured animals. See J. A. Allen ('94) and H. C. Merriam ('95).

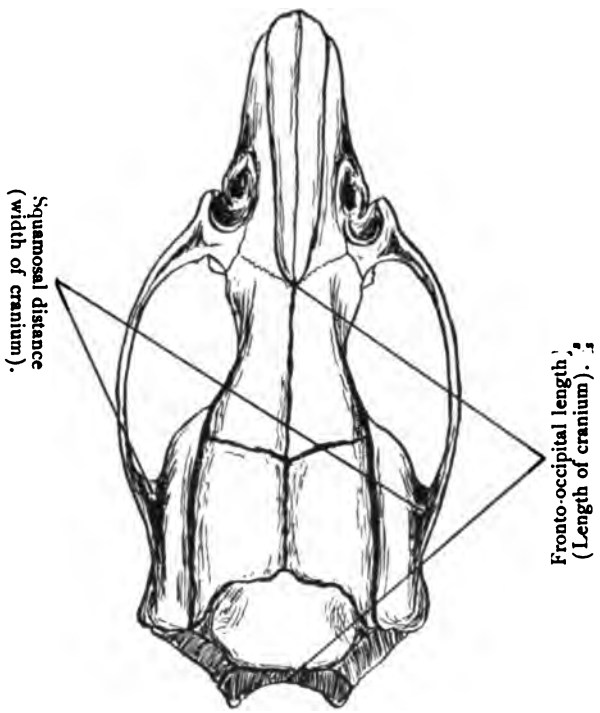


FIG. 3. (× two diameters.) The measurement of frontal-occipital length was determined in the following way:

Since the length measured from the tip of the nose to the posterior end of the inter-parietal bone, is not always equal to the length measured from the tip of the nose to the end of the occipital bone, both measurements were taken. First, the measurement from the tip of the nose to the end of the occipital bone, and second, that from the tip of the nose to the end of the inter-parietal bone. The difference thus obtained, was added to the length of the frontal-interparietal line, and the sum was called frontal-occipital length.

The width of the cranium was determined by taking a maximum width between the two points (right and left) where the zygomatic bones rest on the lateral walls of the cranium.

We conclude therefore, that the albino rats composing the colonies at Chicago and Philadelphia, are similar to *Mus norvegicus* in their bodily proportion, and in their cranial characters. They are however, smaller in size than the specimens of *Mus norvegicus* usually found.

TABLE SHOWING CRANIAL INDEX.

Males.	Cranial Index Average.	Extremes.	No. of Rats Used.
Mus rattus	.60	.58–.62	8
Mus norvegicus	.54	.51–.55	12
Albino rat	.54	.50–.56	12

Nevertheless this form is to be regarded as an albino variety of that species and to be designated *Mus norvegicus* var. *albus* (*oculis rubicundis*).

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EFFECT OF PARTIAL STARVATION FOLLOWED BY A RETURN TO NORMAL DIET, ON THE GROWTH OF THE BODY AND CENTRAL NERVOUS SYSTEM OF ALBINO RATS.

By SHINKISHI HATAI.

[From the Wistar Institute of Anatomy and Biology, Philadelphia.]

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EFFECT OF PARTIAL STARVATION FOLLOWED BY A RETURN TO NORMAL DIET, ON THE GROWTH OF THE BODY AND CENTRAL NERVOUS SYSTEM OF ALBINO RATS.

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IN my previous research (:04) on the effect of partial starvation on the brain of the albino rat, it was shown that in the experimented rats, fed with starch, beef fat, and water for twenty-one days (younger group, rats thirty to forty days old; older group, rats one hundred and fifty to two hundred days old), not only the growth of the brain was stopped, but brain substance was lost, the actual loss from the weight before starvation being, on the average, 4.67 per cent. In such experimented animals, also, the percentage of water in the brain was diminished (79.08 per cent control, and 78.84 per cent experimented) and of the ether-alcohol extracts increased (46.69 per cent control, and 47.61 per cent experimented — Hatai, :04).

As the absolute weight of the brain in the starved group was diminished, and as the relative amount of the extracts was increased, the writer inferred that the protein substances had been most affected. The total loss in the body weight in the experimented rats at the end of twenty-one days was 29.7 per cent.

Having established the fact that the brain is definitely modified as the result of partial starvation for twenty-one days, the next question was:

Can the nervous system thus affected recover when the animal is returned to a normal diet? The present research was undertaken in order to answer this question.

Altogether thirty-two rats, representing seven litters, were used. One half of each litter was subjected to partial starvation, and the other half used for control. The rats were so grouped that at the beginning of the observation the average body weight in the control group balanced that in the experimented. As soon as the young

albino rats reached thirty days of age, the experimented groups were fed with starch (Oswego cornstarch) and water alone,[1] while the control groups were fed with the usual diet, — corn, cabbage, milk, bread, meat, etc. The supply of food for both groups was abundant. After twenty-one days of starvation the experimented rats were at once put on the full normal diet, and then fed for the succeeding one hundred and forty-nine days with the same diet as was given to the control group. When the rats became two hundred[2] days old, they were killed, and the weight of the central nervous system, percentage of water, and percentage of the ether-alcohol extracts were determined. The dissection of the rats, and the procedure for the determination of weight, etc., were carried out with all the precautions used in the previous experiments.

Body weight. — As is shown in Table I, the initial weight of the experimented male rats was slightly greater (6.5 per cent) than that of the male controls, while that of the experimented females was slightly less (5.5 per cent) than that of the female controls. As the result of the partial starvation, the loss in the body weight of the experimented group was 24 per cent in the male, and 21 per cent in the female, while the increase in the control group for the same period amounted to 44.2 per cent in the male, and 46.4 per cent in the female. Thus, after twenty-one days (*e. g.*, at the age of $30 + 21 = 51$ days), the difference in the weight between the control and experimented groups became very large, amounting to 55 per cent in the male, and 60 per cent in the female, the weight of the control group being taken as the standard.

In my previous research the loss in the weight (younger series, thirty to forty days old) after twenty-one days of starvation was even greater; the average for both sexes being 32 per cent, as contrasted with 23 per cent in the present research. It must be remembered, however, that in the earlier experiments the rats were slightly older. Thus the greater loss in weight in the previous case was, perhaps, in some measure due to the reduction of fat.

After twenty-one days of starvation the experimented rats showed marked changes, the vertebral spines were evident, the trunk slightly curved, the pink color had entirely disappeared from the soles of the feet as well as from the external ear, the eyelids were partly closed,

[1] The beef fat was omitted in the present experiment.

[2] It was found in our laboratory that the albino rats of two hundred days old are fully matured. The sexual maturity takes place at about seventy days of age.

and movements were rather unsteady. The rats in this state were removed to the other cages and at once given a full normal diet. The rapidity of recuperation was surprising, as it took only three or four days for them to return to their initial weight.

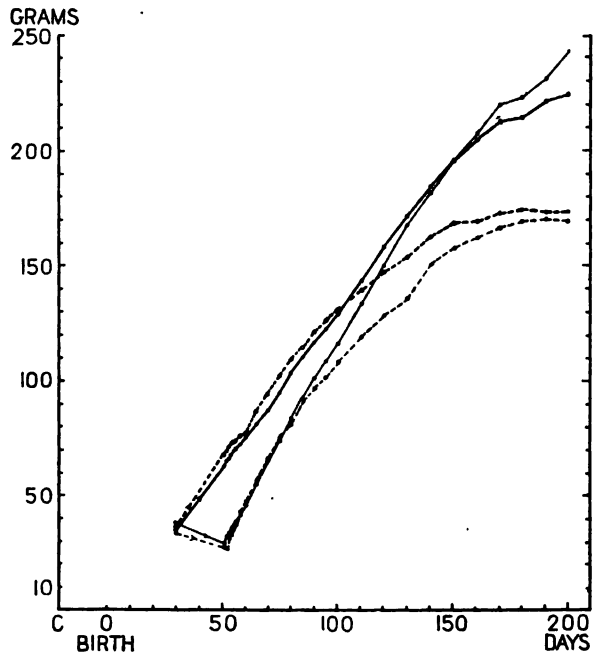

FIGURE 1. — Curves showing the body weights of albino rats at different ages. *C*, conception, and *O*, the date of birth twenty-one days after conception. •—— • Males Starved. o·····o Female Starved. ——— Male Control. o····o Female Control.

It was naturally asked whether or not the mere distention of the stomach was responsible for the rapid recovery in the body weight. A careful examination, however, showed that this was not the case. Within three to four days almost all the evidences of disturbed nutrition, enumerated above, entirely disappeared. The pink color reappeared in the soles of the feet and the external ear; the eyes were held widely opened, the trunk became straight. The movements, however, were not so steady or so active as in the normal rats. An interpretation of this rapid recovery is postponed until the composition of the urine during such a period, as well as some other points, have been determined.

The daily increase or decrease in weight is shown in Fig. I. At

TABLE I.

Body weight.			Weight of		Percentage, water.	
Initial.	After 21 days.	Final.	Brain.	Sp. cord.	Brain.	Sp. cord.
					per cent.	per cent.
23.6	60.3	211.5	1.7810	0.5520	77.36	69.61
25.6	48.6	233.8	1.8030	0.5348	77.79	70.26
27.2	56.0	205.0	1.7988	0.5614	77.79	69.50
28.2	54.0	188.4	1.7214	0.5330	77.13	69.60
31.4	55.6	228.8	1.9264	0.5894	77.28	69.90
36.2	59.8	199.2	1.7641	0.5168	77.60	70.54
42.7	76.4	223.4	1.8847	0.5814	77.27	69.38
43.2	74.2	211.2	1.8634	0.5415	77.80	69.56
58.6	83.0	318.4	2.1862	0.6548	77.51	69.06
....	....	....	....	....	....	....
Averages.						
35.2	63.1	224.4	1.8587	0.5628	77.50	69.71
Control Rats (Females).						
24.2	72.4	161.2	1.6644	0.4770	77.60	70.10
26.5	59.0	141.3	1.6864	0.4866	77.44	69.21
26.8	54.8	154.2	1.7018	0.4864	77.65	69.10
42.5	75.8	188.8	1.8664	0.5582	77.34	69.13
46.2	74.6	201.6	1.9063	0.5834	77.39	69.22
51.6	70.2	188.2	1.9272	0.5587	77.58	69.62
....	....	....	....	....	....	....
Averages.						
36.3	67.8	172.6	1.7905	0.5250	77.50	69.40

TABLE I (*Continued*).

Percentage, water.		Weight of		Body weight.		
Sp. cord.	Brain.	Sp. cord.	Brain.	Final.	After 21 days.	Initial.
per cent.	per cent.					
71.06	78.05	0.5108	1.7930	199.7	20.8	27.2
69.97	77.84	0.5482	1.7507	196.3	22.8	27.5
70.95	77.90	0.4978	1.6138	194.0	22.2	28.8
69.79	77.74	0.5714	1.9514	228.7	23.8	31.1
70.20	77.82	0.5940	2.0681	262.0	25.0	32.0
70.47	77.68	0.5460	1.7696	215.8	26.4	32.0
69.97	77.91	0.5902	1.8779	256.0	30.4	40.8
69.66	77.39	0.5988	1.9493	281.0	32.2	43.9
69.05	77.52	0.5926	1.9774	259.0	38.2	52.2
69.46	77.61	0.7162	2.1154	327.0	42.4	60.6
Averages.						
70.05	77.75	0.5766	1.8866	242.0	28.4	37.6
Experimented Rats (Females).						
71.11	77.90	0.4120	1.5614	130.0	20.2	24.0
70.81	78.01	0.4520	1.5696	138.2	21.4	24.6
69.56	77.64	0.5152	1.6800	176.0	28.9	36.1
69.46	77.91	0.5804	1.8472	198.8	29.7	36.9
70.85	77.82	0.4858	1.6372	152.0	26.3	37.8
69.92	77.60	0.5593	1.8811	181.0	32.4	40.0
69.21	77.41	0.5574	1.8608	199.0	30.0	40.9
Averages.						
70.10	77.75	0.5089	1.7196	167.8	27.0	34.3

the end of two hundred days the final weight in the experimented and in the control was 242 and 224 gm. respectively, in the case of the males, and 173 gm. and 168 gm. in the case of the females; that is, the experimented male rats were slightly heavier (7.4 per cent) than in the controls, while in the case of the females the control rats were the heavier (2.5 per cent).

The comparison between the initial and final weight is shown in Table II.

TABLE II.

	Body weight.				Ratio between initial and final.
	Initial.	After 21 days.	Final.	Total gain.	
Male, controls . . .	35.2	63.1	224.4	189.2	1 : 6.37
Male, experimented . .	37.6	28.4	242.0	204.4	: 6.43
Female, controls . . .	36.3	67.8	172.6 [1]	136.3	: 4.75
Female, experimented .	34.3	27.0	167.8 [1]	133.5	: 4.89

From the above it is seen that the absolute gain in weight is slightly greater in the experimented males, and slightly smaller in the experimented females, as compared with their controls. The ratios, however, show that the relative gain is approximately the same in both experimented and control groups, although slightly greater in the case of the experimented rats in both sexes.

I therefore conclude that so far as the body weight is concerned, the experimented rats have completely recovered from the effect of the twenty-one days of partial starvation.

It has been frequently observed by different investigators — Coudereau ('69), Pagliani ('79), and others — that the growth of the body in recuperation is very rapid in the children whose growth has been temporarily disturbed by illness or other unfavorable conditions. This observation is well supported by the present experiments. As is shown in Table I, as well as in Fig. I, the recovery in the weight is most astonishing, especially during the first three or four days, within which time the starved rats regain the weight lost during the twenty-one days of starvation. Later the increase in weight is very steady, though not as rapid as during the first few days, until the rat has reached the age of one hundred and fifty days, and after this age increase in weight is relatively slow.

What will happen to such rats during the later portions of the

[1] The body weight in both control and experimented is small for the age.

span of life has yet to be determined in order to answer the question whether this partial starvation in early life has any influence either on longevity or the onset of old age.

Weight of brain and spinal cord. — After the body weight had been taken, the brain and spinal cord were removed separately, and their weights were carefully determined. The spinal cord was

TABLE III.

	Body weight.	Encephalon.	Sp. cord.
Male, controls	224	1.8587	0.5682
Male, experimented . .	242	1.8866	0.5766
Female, controls . . .	173	1.7905	0.5250
Female, experimented .	168	1.7196	0.5089

severed from the encephalon at the tip of the calamus scriptorius. The spinal roots were cut off as close to the cord as possible. The results obtained are seen in Table III.

As it stands, the weight of the central nervous system in the experimented male is heavier, and in the experimented female lighter, when compared with the corresponding controls. Since the brain weight is closely correlated with the body weight, we should expect a heavier brain weight in the heavier individuals, and therefore it is desirable to determine whether or not the brain weights found in the experimented rats correspond with the given body weights. Dubois ('98) found that in man, at maturity, the brain weights were related as the fourth roots of the body weights.

Dhéré and Lapique ('98) have determined a like relation for dogs of different sizes where they found a good accordance between the observed and calculated results. An application to the present data of this law given by Dubois for the human brain weights, shows that in the case of the males 1.8866 gm. of brain in the experimented males should correspond to the body weight of 238.3 gm., instead of 242 gm., when the relations in the control group are used as a standard. The difference (3.7 gm.) is too small to be considered significant. It is therefore quite safe to conclude that so far as the relation between brain weight and body weight in the male is concerned, the starvation effect has been completely removed. In the case of the female we have some difficulty in applying Dubois' law, since the brain weight in the control group is too high [1] for the given body weight. Accord-

[1] This fact was determined by examining some ten records preserved in our archives, for female rats having a body weight of about 173 gm.

ing to Dubois' law, the figures for the control group being taken as the standard, a brain of 1.7196 gm. found in the experimented group should correspond to body weight of 147.3 gm., instead of 168 gm., as observed. It was found that according to our standard curve 1.7196 gm. of brain weight there corresponds to the body weight of 162 gm., or 6 gm., less than that observed. This difference is negligible in view of the variability in this relation, and therefore it seems

TABLE IV.

	Percentage of water.	
	Encephalon. per cent.	Sp. cord. per cent.
Male, controls	77.50	69.71
Male, experimented	77.75	70.05
Female, controls	77.50	69.40
Female, experimented	77.75	70.10

safe to conclude that in the experimented female group partial starvation has not permanently modified the relation of brain weight to body weight.

In regard to the weight of the spinal cord, it is clearly shown that the cord also follows body weight in the same manner as does the brain, but the details have not been worked out.[1]

Percentage of water in the central nervous system. — From Table IV it is clearly seen that percentage of water in both encephalon and spinal cord is higher in the experimented rats than in the control rats.

On the average, the difference between the experimented and control is 0.25 per cent in the encephalon of both sexes, and 0.34 per cent in the male spinal cord, and 0.70 per cent in the female, always in favor of the experimented groups. Although the difference shown is small, nevertheless it is significant, since the difference appears in all but one instance when the representatives of the same litter are compared.

From unpublished observations in this laboratory, it has been concluded that during normal growth the percentage of water in the

[1] An application of Dubois' law to the weight of the spinal cord shows that, when the relations in the control group are used as a standard, 0.5766 gm. of spinal cord in the experimented males correspond to the body weight of 247.4 gm. (difference 5.4 gm.), and 0.5089 gm. of the experimented female spinal cord to 152.8 gm. of the body weight (difference, 15.2 gm.). These calculated values of the body weight agree with those calculated from the brain weight, indicating that the relation of the spinal cord to the body is similar to that of the brain.

TABLE V.

Litter 1.					
Control Rats.			Experimented Rats.		
Body weight.	Percentage, water.		Percentage, water.		Body weight.
	Brain.	Sp. cord.	Sp. cord.	Brain.	
	per cent.	per cent.	per cent.	per cent.	
223.4 M.	77.27	69.38	69.66	77.39	M. 281.0
188.8 F.	77.34	69.13	69.35	77.41	F. 199.0
Litter 2.					
318.4 M.	77.51	69.06	69.46	77.61	M. 327.2
201.6 F.	77.39	69.22	69.46	77.91	F. 198.8
188.2 F.	77.58	69.22	69.90	77.60	F. 181.0
			69.56	77.64	F. 176,0
Litter 3.					
211.2 M.[1]	77.80	69.56	69.05	77.52	M. 259.0
Litter 4.					
199.2 M.	77.60	70.54	69.97	77.91	M. 256.2
			70.47	77.68	M. 215.8
			70.85	77.82	F. 152.0
Litter 5.					
233.8 M.	77.79	70.26	70.20	77.82	M. 262.0
228.8 M.	77.28	69.90	71.06	78.05	M. 199.7
205.0 M.	77.79	69.50	70.95	77.90	M. 194.0
188.4 M.	77.13	69.60	71.11	77.90	F. 130.0
154.2 F.	77.65	70.10			

[1] Litter 3 alone is an exceptional case where the percentage of water in the control is higher than in the experimented rat.

TABLE V (*continued*).

Litter 6.					
Control Rats.			Experimented Rats.		
Body weight.	Percentage, water		Percentage, water.		Body weight.
	Brain.	Sp. cord.	Sp. cord.	Brain.	
	per cent.	per cent.	per cent.	per cent.	
211.5 M.	77.36	69.61	69.79	77.74	M. 228.7
141.3 F.	77.44	69.21	69.97	77.84	M. 196.3
Litter 7.					
161.2 F.	77.60	69.10	70.81	78.01	F. 138.2

nervous system of the rat is mainly a function of its age, and is but slightly modified by the weight of the central nervous system or of the size of the body (Donaldson). In these experiments, however, the relation of age to the nervous system is considerably modified in the experimented rats, since not only the growth of the nervous system has been completely stopped for twenty-one days, but the percentage of water was diminished (79.08 per cent control, and 78.84 per cent experimented — Hatai, :04) by this treatment. Therefore the higher percentage of water found in the central nervous system of the experimented rats after recovery may mean one of two things: (1) As the result of partial starvation, the destructive process which has been traced (see previous paper, Hatai, :04,) might produce a diminution of nerve tissue, and the relatively higher content of water might be due merely to an accumulation of fluid occupying the spaces which had been so developed; (2) It may indicate a much more active metabolic process, and be an indication of recuperative activity.

The second assumption seems the more probable, since the weight of the central nervous system was normal in relation to the body weight, indicating that there was not a mere accumulation of the fluid occupying newly formed spaces, for this would have tended to reduce the brain weight.

Recently Watson (:05) made observations on the effect of the bearing of young upon the body weight and the weight of the central nervous system of female albino rats, where he has shown that the brain and spinal cord of the mated individuals contained a slightly higher percentage of water.

He gives the following percentage values:

	Percentage of water.	
	Brain. per cent.	Sp. cord. per cent.
Mated (average — 8 rats) . . .	77.47	68.51
Unmated (average — 10 rats) . .	77.37	68.29

Watson's experiments may be considered analogous to mine, as he is also dealing with animals that have passed through a period of partial starvation, since during lactation the mother loses body weight to quite an extent (Minot, '91; Watson, :05). Although it is impossible at the present moment to make any definite statement, our observations on partial starvation suggest that the higher percentage of water found in the rats bearing young might have been produced as the effect of temporary retardation of the growth of the nervous

TABLE VI.

	Percentage of extracts.	
	Encephalon. per cent.	Sp. cord. per cent.
Male, controls	50.61	68.83
Male, experimented	50.36	68.54
Female, controls	49.56	68.87
Female, experimented	49.16	68.40

system followed by recovery. This can be determined only by a study of the nervous system during the period of greatest retardation in rats bearing young.

It remains, moreover, to be determined whether or not our experimented rats will in this respect recover entirely from this condition which is produced by the twenty-one days of partial starvation.

In the normally grown rats the percentage of extracts in the nervous system is also a function of age, and is inversely related to the percentage of water (Donaldson). Thus the determination of the percentage of extracts would furnish us further evidence as to the normal condition of the growth of the nervous system with respect to age and to the percentage of water.

It is clear from the preceding Table VI that the percentage of ex-

tracts is less in the experimented group in both encephalon and spinal cord than in the control. In the case of the encephalon 0.25 per cent in the male and 0.40 per cent in the female, and in the case of the spinal cord, 0.29 per cent in the male and 0.47 per cent in the female are in favor of the control rats. These are the results that one would expect. It is therefore concluded that the amount of extract, as compared with the residue, found in the nervous system of the experimented rats is normal with respect to the age and percentage of water in that organ.

Conclusions.

From what has been presented, the following conclusions are drawn:

(1) So far as the weight of the body and central nervous system are concerned, the effect of a twenty-one day period of partial starvation on albino rats thirty days old is eventually completely compensated.

(2) The chemical composition of the brain and spinal cord is, however, not entirely free from the effect, as is indicated by the higher percentage of water, and lower percentage of ether-alcohol extracts, in the experimented rats, as compared with the controls.

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A Study of the Diameters of the Cells and Nuclei in the Second Cervical Spinal Ganglion of the Adult Albino Rat

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With Four Figures

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A STUDY OF THE DIAMETERS OF THE CELLS AND NUCLEI IN THE SECOND CERVICAL SPINAL GANGLION OF THE ADULT ALBINO RAT.

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WITH FOUR FIGURES.

INTRODUCTION.

It is generally believed that the spinal ganglion contains several types of nerve cells which can be morphologically differentiated from one another. The varieties of cells whose existence in the ganglia have been repeatedly confirmed are: (1) cells with a T- or Y-shaped division of the processes. Such cells are considered to be most abundant and to be both large and small in size. (2) DOGIEL'S cells of second type, multipolar cells, and (3) multipolar cells which resemble in shape and structure sympathetic ganglion cells. The most complete classification is based on the study of methylene blue preparations. In a general way the presence of the several varieties of cells in the ganglia may also be demonstrated in ordinary paraffine sections treated with any of the basic dyes followed by a counter-stain. In such preparations one can easily distinguish cells of different sizes as well as those exhibiting different arrangements of the stainable substance. These two characters, size and arrangement of stainable substance, have been used as a criterion by several investigators in order to classify these cells.

By this method LUGARO ('96) distinguishes in the dog five different varieties of the spinal ganglion cells, LENHOSSÉK ('96) in the human spinal ganglion distinguishes three varieties, COX ('98) in the spinal ganglion of the rabbit, two main varieties, and the author ('01) using the same criterion has distinguished three varieties in the spinal ganglion of the albino rat. It is my

intention later to analyse in detail all these classifications and at the present moment it is merely necessary to call attention to the fact that in the spinal ganglion several varieties of cells have been distinguished from one another. Can all these cells of different varieties be considered as belonging to a single class or are there really several types of cells composing the spinal ganglion? In other words, a frequency of distribution of all these cells based on their sizes[1] should give us more than one mode if there were more than one type of cell involved. If but one mode appears we have good ground to conclude that all these cells, though differing in size as well as in structure, may be considered from the standpoint of size, as members of a homogeneous population. The differences in structure are for the moment neglected and must form the subject of a special study.

MATERIAL AND TECHNIQUE.

For the present investigation the second cervical spinal ganglion of the adult albino rat was employed. The second cervical ganglion was purposely selected since through the investigation of RANSON ('06) we have already some numerical data in regard to this particular nerve.

The second nerve with ganglion was removed from right side of a healthy male having a body-weight of 194 grams and was fixed with osmic acid. Following the usual procedure the sections of the ganglion were cut 12 micra thick and mounted in series. Three sections from the middle of the entire series of 80 sections and three sections from midway between the middle and end on both sides, thus making altogether nine sections, were chosen. These nine sections were selected for the measurement of the cells and nuclei on the assumption that the cells of the different sizes were uniformly distributed and consequently that the nine sections would adequately represent the total cell "population" of the ganglion.

The measurements obtained from each cell and its nucleus were recorded on a separate card. In every case two maximum diameters at right angles to each other were determined for both cell-body and nucleus by means of the ocular micrometer. The

[1] Although this point could be tested also from the standpoint of the structure, nevertheless it is very difficult to obtain numerical data in terms of the structure, suitable for biometric treatment.

values of the two diameters thus obtained were multiplied together and the square root of the product was called "calculated diameter" of the cells and nuclei. Of course every section of a cell which possessed a distinct nucleus and nucleolus was measured from the nine sections and altogether 1108 such cells were found. The 1108 cells and nuclei thus measured were arranged according to the magnitude of the "calculated diameters" and the frequencies of the variates were determined as shown in the correlation table (see p. 490). For grouping the variates I have selected 2 micra as the unit for the cell-body and 0.65 of a micron for the nucleus. As will be seen later, small differences in the value of the unit do not produce any significant change in the final results, and therefore it is advisable to select some integral number for convenience in computation.

ANALYTICAL CONSTANTS AND FREQUENCY DISTRIBUTION.

I shall first discuss the frequency distributions of the cell-bodies and nuclei. The fundamental analytical constants necessary for such discussion are given in the following table:

TABLE I.

No. of measurement.	Cell-body.	Nucleus.	Skewness.	Cell-body.	Nucleus.
	1108	1108		.4081± .024	.1734± .024
μ_3	27.1627	9.5254	Modal divergence	1.3558± .006	.4918± .002
μ_4	446.9891	233.3303	Standard deviation	6.6448± .952	1.8457± .026
β_1	.5486	.1731	Mean	23.3356± 1.346	10.9523± .037
β_2	3.6687± .099	3.5889± .099	Mode	21.9798	10.4605
$\sqrt{\beta_1}$	.7407± .052	.4161± .028	Coef. of variation	28.4749± 0.4398	16.8521± 0.2482
β_2-3	.6687	.5889	Coef. of correlation	.8616± .006	
κ_1	−.3084	.6585	Lower and upper ends of ranges	7.5844 60.9693	
κ_2	−1.5179	.8248	Type of curve	I	IV

It has been shown by PEARSON ('05) that in order to fit a given distribution of frequency to a Gaussian probability curve

the following conditions, within the limit of random sampling, must be fulfilled:

$$\sqrt{\beta_1} = 0; \beta_2 - 3 = 0; \tfrac{1}{2}\frac{\sqrt{\beta_1}(\beta_2 + 3)}{5\beta_2 - 6\beta_1 - 9} = 0; \text{and } d, \text{modal divergence} = 0.$$

By examining the analytical constants given in Table I, it is seen that all those constants for both the cell-bodies and nuclei are considerably greater than zero even when their respective

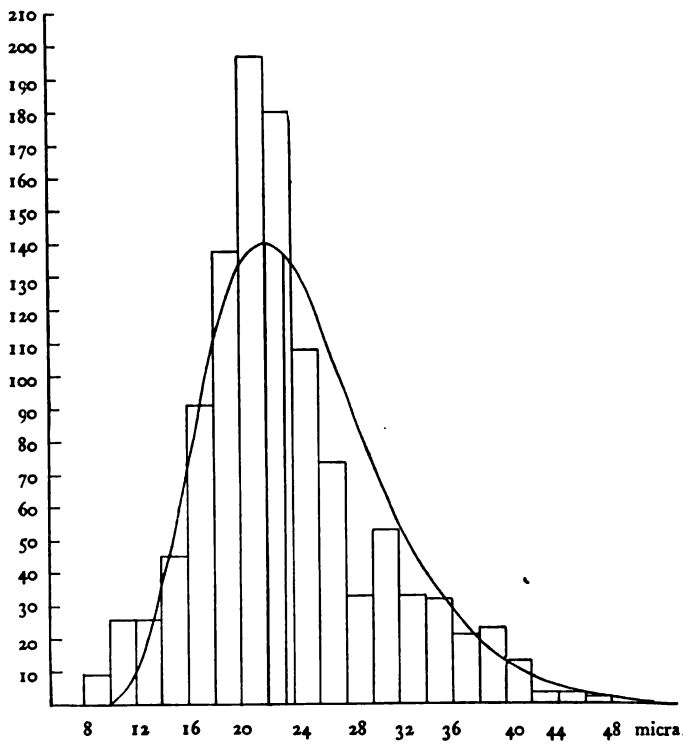

FIG. 1. Frequency polygon and fitted curve for variation in the diameters of the cell-bodies.

probable errors are considered. This at once leads to the conclusion that in both cases the frequency distribution can not be represented by the normal curve. Furthermore a considerable deviation of those constants from zero, *i. e.*, skewness, as well as modal divergence, indicates that they can never be represented by any other symmetrical curve since the deviation in excess and

defect are not equally probable. It is therefore evident that n order to represent the data in hand we must find a curve which is able to represent the odds against any given deviation.

It has also been shown by PEARSON ('95, '01) that the assignment of a given distribution of frequency to any one of the six

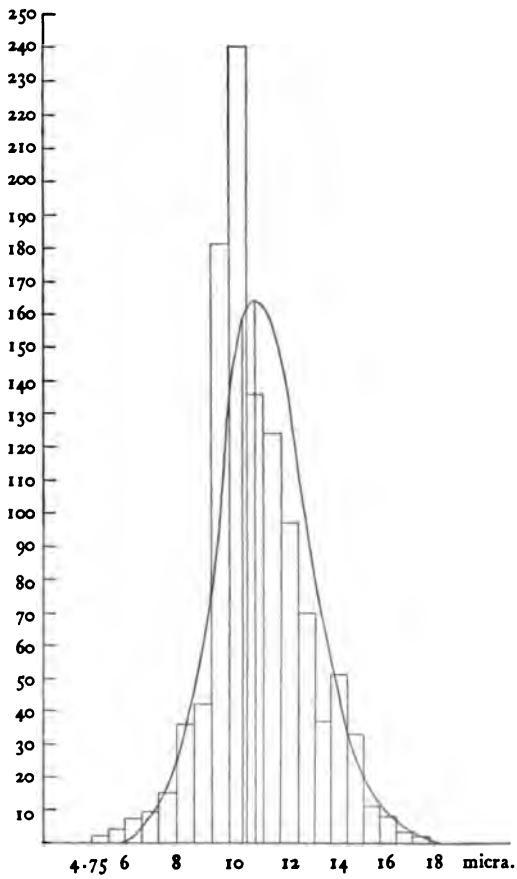

FIG. 2. Frequency polygon and fitted curve for variation in the diameters of the nuclei.

types of his skew curves depends on the value of the analytical constants κ_1; κ_2; β_1 and β_2. As is shown in Table I, in the case of the cell-body we have the following relations:

$$\kappa_1 < 0; \kappa_2 < 0 \text{ and } \beta_1 > 0$$

These three conditions satisfy PEARSON's skew frequency curve of Type 1, while for the nuclei we have

$$\kappa_1 > 0; \beta_1 > 0; \beta_2 > 3; \text{and } \kappa_2 > 0 \text{ and } < 1,$$

which calls for PEARSON's curve of Type 4.

The frequency distributions and their fitted curves are shown graphically in Figs. 1 and 2. The equations for the curves are:

For the cell-bodies (Type 1)[2]

$$y = 140.0657 \left(1 + \frac{x}{6.2286}\right)^{3.6107} \left(1 - \frac{x}{62.3049}\right)^{35.6365}$$

origin at mode.

For the nuclei (Type 4)

$$y = 13.0825 \left(Cos\,\theta\right)^{24.0118} \varepsilon^{11.2038\theta}$$

origin at 7.4341 micra,

$$x = 11.6002 \ tan\,\theta$$

Examining Figs. 1 and 2 we see at once that the theory and observation do not agree at all well. The theoretical curve in both cases considerably underestimates the observed ordinates for the smaller values of variates x, and overestimates the same for the larger values of x. The degree of deviation between the observed and theoretical curves is most pronounced at or in the neighborhood of the mode. This unexpected results forced the writer to reinvestigate the following points:

1. Since the spinal ganglion contains various sized cells it may be possible that these cells are not uniformly distributed from

[2] Original formula of Type 1 is given by

$$y = y_0 \left(1 + \frac{x}{a_1}\right)^{m_1} \left(1 - \frac{x}{a_2}\right)^{m_2}, \text{ where } y_0 = \frac{\alpha}{b} \cdot \frac{m_1^{m_1} m_2^{m_2}}{(m_1 + m_2)^{m_1 + m_2}} \cdot \frac{\Gamma(m_1 + m_2 + 2)}{\Gamma(m_1 + 1)\Gamma(m_2 + 1)}.$$

and for Type 4

$$y = y_0 (Cos\,\theta)^{2m} \varepsilon^{-\nu\theta}, \text{ where } y_0 = \frac{\alpha}{a} \cdot \frac{\varepsilon^{\frac{1}{2}\nu\pi}}{\pi \int_0 Sin^{r}\theta\, \varepsilon^{\nu\theta} d\theta}.$$

section to section. In other words, some sections may contain relatively more cells having larger or smaller diameters, therefore the nine sections selected might not give a proper representation of the entire cell population and therefore the 1108 cells measured might not constitute a real random sampling of the entire population.

2. The disagreement between the theory and observation may be due to an improper selection of the unit for grouping the variates. If so, it may be improved by taking some other unit.

3. The two curves may agree more closely if the uncorrected or raw moments about the mean were used in determining various analytical constants.

4. The spinal ganglion cells may not represent a homogeneous population, but a mixture of various groups of elements. If so, dissection of the frequency curve into several components should give a better agreement.

The question contained in point 1 has been answered in the following way: the nine sections were divided into three series, each represented by three sections. For the series 1, one section was taken from the middle and one from the midway between the middle and the extremes on both sides, while for the series 2, the three sections which lie to the right of the three sections of the series 1 and for the series 3 those which lie toward left. The percentage values of these three series just mentioned were plotted separately, the same unit of course being used for each series. Comparison shows that these three curves agree with each other in every minor detail, and therefore with the original curve, too. This means that the cell-bodies of different sizes are uniformly distributed, otherwise the three curves should not agree so closely. Therefore the 1108 cells here measured can be regarded as giving a true representation of the entire cell-population. The disagreement found between the theoretical and observed curves is consequently not due to a lack of uniformity in distribution.

The question contained in point 2 was also answered by taking three different units for the cell-bodies; 1, 1.8, 2 micra; and one different unit for the nuclei, 1 micron, and comparing the new results with those already obtained. It was found that these variations in the units did not make any significant alteration in curve. This proves then the difficulty is not due to an improper choice of the unit.

As to point 3, I have treated the data for the cell-bodies, using the uncorrected moments, but without effecting any improvement on the results.

Finally I have tried to split the observed frequency curve (point 4) into two normal curves according to the method given by PEARSON ('94). After laborious calculations it was found that the present data can not be split. The reason for this conclusion is omitted since it needs an elaborate mathematical presentation. The result shows however that there is not the slightest indication of separate groups in the cell-population.

Therefore the cause of disagreement must depend on other conditions than those already enumerated.

After failing to obtain in this way a reasonable explanation for the considerable deviations between the observed and theoretical

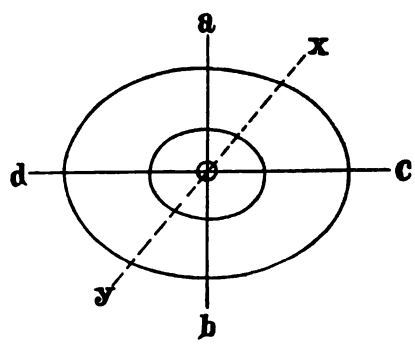

FIG. 3. Diagram of the spinal ganglion cell containing nucleus and nucleolus.

curves, it occurred to me that the explanation might be found in the method of sectioning and measurement. In order to make clear the relations existing within the ganglion let us suppose that 8000 spinal ganglion cells of various forms (from spherical to oblong) are thoroughly mixed in an ovoid receptacle. This is then cut into 80 slices of equal thickness. The entire series is sampled by taking three slices from the middle and three slices from the midway between the middle and extremes on both sides. Thus nine slices are selected for examination. The slices of the ganglion which we have examined in this way contained 1108 cells. Under these conditions the knife cuts the individual ganglion cell

in various planes. In some cases the knife makes a right angle with the longest axis (*dc*, Fig. 3) of the cell-body and some cases with the shortest axis (*ab*). In the remaining cases the angles will always be less than 90°. It must be remembered that the 1108 cells counted are those which contain both nucleus and nucleolus, and therefore it is assumed that the knife always passed through the approximate center of the cell-body. The chance that the knife will make a right angle with shortest axis[3] must however be very small compared with a failure. Whichever plane we cut, as long as the knife passes through the center, the diameter (*ab*) is constant and therefore the product of the diameters varies directly with the changes in the longer. But (*cd*) is the maximum diameter and its length diminishes as the axis moves from the original position toward the axis (*ab*). As soon as it reaches (*ab*) it becomes minimum. As was stated already, there are more chances for the knife to pass through somewhere between the two points (*a*) and (*c*) than to cut through (*c*) itself. If therefore we determine the two diameters of the cell from the cut surface and the square root of their product is taken as the mean or "calculated diameter" of the given cell, the final value thus obtained will often be less than it should really be, because $\sqrt{ab \times dc}$ is always $> \sqrt{ab \times xy}$, where (*xy*) is any arbitrary line between (*a*) and (*c*). But as was stated already, the sections of the smaller cells are more nearly circular in outline. Therefore the mean diameters thus obtained may represent nearly the true value in the case of the smaller cells but less nearly, in the case of the larger cells which have become ovoid. From this it will be clearly seen that the frequency curve of the diameters of the ganglion cells based on the square roots of the product of the observed diameters can not represent the true frequency.

As to the range of the diameters, the maximum "calculated diameters" found may be considered to be the true value since there is at least one chance that the knife could pass through the longest axis while on the other hand the observed minimum diameter may be somewhat less than the true minimum diameter since there is a tendency for the diameter of those cells which are in any degree ovoid to be made smaller. Consequently if the values

[3] The maximum size of the cell is obtained only when the knife makes 90° with the axis (*ab*), provided it also passes through the center of the cell.

found for the small cells were nearly right but those found for the large cells were less nearly right it is evident that we should expect to find more cells showing smaller diameters than are actually present in the nine slices examined. This leads at once to the conclusion that the ordinates representing the cells of the small diameters must be compounded of two heterogenous elements; *the true small cells plus those cells which are artificially made smaller* by the method of section. In the same way the ordinates for the larger cells will be compounded of the measurements on *the larger cells minus those which were artificially made smaller.* Thus we shall find an excess of cases towards the smaller value of x and a deficit towards the larger value of x. This is just what we have observed. It was found, when the observed polygon was compared with the theoretical curve that the latter considerably underestimated the observed ordinates which correspond to the smaller values of x and overestimated the observed ordinates which correspond to the larger values of x. The facts mentioned above indicate that the deviations of the observed polygon from the theoretical curve are mainly, if not entirely, due to the method of section. The greater observed excess found in the neighborhood of the mean or mode is interesting, since it may be assumed that up to the neighborhood of the mean or mode the cells remain nearly spherical while beyond this region the increase in size is accompanied by a change in shape.

If my argument is correct, then we should expect the greater percentage deviation of the two diameters to appear more frequently towards the larger abscissal values. Although it would seem at first easy to test such hypothesis by taking the percentage deviation from the averages corresponding to the different abscissal values, nevertheless in practice we meet considerable difficulties. Any one who is familiar with the sections of the spinal ganglia prepared by the usual fixation methods, will recall that there are present a number of *small* cells with very unequal diameters. Such cells are most abundant along the periphery of the sections. The general outline of such cells is either rectangular, instead of curved, or the opposite boundaries are represented by nearly parallel lines. The cause of the deformation may be attributed to a shrinkage of the capsule of the spinal ganglion itself. On account of the presence of such deformed cells a mere comparison of the

percentage deviations of the two diameters is unsatisfactory. Although it may not be a conclusive test, yet remembering that the spherical or nearly spherical cells should occur more frequently either towards the negative side or at the mode than towards the positive side, a determination of the relative frequency of such spherical cells, or the cells with nearly equal diameters, may be employed. Under the circumstances I think this is the only feasible method of testing this point. From an examination of original data, it has actually been found that such spherical or nearly spherical cells diminish with the increasing calculated diameter and increase with a diminishing calculated diameter. Even without any further test we cannot doubt from the theoretical standpoint that the method of section diminishes the diameter of the large cells, thus artificially increasing the frequencies of the small cells.

If this fact just mentioned is accepted, the conclusion follows that the theoretical curves may be considered as satisfactory representations under the circumstances and also may be considered much truer representations of the frequency distributions of the cell-bodies and nuclei than that shown by the actually observed data.

Since the curve of Type I has limited range in both directions, we find from the constants that

$$\text{lower limit of range} = 7.5844\mu$$
$$\text{and upper limit of range} = 60.9693\mu,$$

while the observed limits are 7.8 micra and 47.4 micra, respectively. We see therefore that the theoretical lower limit agrees very closely with the observed, while the upper limit in the theory is considerably higher than that of the observed. But that this upper value may not be entirely improbable is indicated by my previous work ('02) on the spinal ganglion cells where I find cells in the fourth cervical spinal ganglion of the adult albino rat as large as 52.7 micra. This figure just given is the average for the three largest cells observed, therefore one or two individual cells must be still larger. Nevertheless it is not necessary to assume that these cells are the largest which could be found. This fact indicates that there is a tendency at least to approximate the values given by the theoretical curve.

MEAN, STANDARD DEVIATION, AND COEFFICIENTS OF VARIATION.

In the equation $A = \frac{\Sigma (V. f)}{n.}$, where A represents the mean, it will be clearly seen that the absolute value of mean (A) varies directly according to the greater or smaller number of frequencies associated with the smaller or greater values of V, as long as "n," the total number of variates, is constant. We have demonstrated above that the number of the observed frequencies of V for both cells and their nuclei cannot be considered as the true frequency owing to the method of section. The true frequencies for the smaller values of V should be the observed frequency minus those cells which have been transferred from the group of large cells, while for the larger values of V it should be observed frequency plus those cells which have been thus transferred. Consequently the mean values actually found for the cell-bodies and nuclei must be considered as smaller than they should actually be. However we cannot determine at the present moment how large the true mean values should be, owing to the difficulty of determining the number of the cells and nuclei which are assumed to have been transferred. On the other hand, the values for the standard deviation and for the coefficients of variation in the present case should be smaller than those found, since following an increase in the frequencies towards the larger values of V the resulting frequency distribution would become more regular than they are shown to be by the observed polygons and consequently the mean square deviation would become smaller. Diminution in the mean square deviation causes a reduction in the value of the standard deviation and consequently in the value of the coefficients of variation. As a matter of fact, we found the value for the standard deviation as well as the coefficients of variation decidedly larger when compared with apparently more variable characters. For example PEARL ('05) found the coefficient of variation in Paramecium from 8 to 9 per cent and in Arcella 10 per cent (PEARL and DUNBAR, '03) while in the present case that of the cell-body is as high as 28 per cent and that of the nucleus 17 per cent. Although we have not as yet any available data with which directly to compare our own, nevertheless our own values appear too great when they are compared with the coefficients of variation obtained from the measurement of highly variable organs like the weight of the

liver (21 per cent, GREENWOOD, '04), weight of the body (10 per cent, PEARSON, '97), weight of the heart (18 per cent, GREENWOOD, '04), etc. I therefore corrected the values of the mean, standard deviation, and coefficients of variation, assuming that the theoretical curves represent more nearly the true distribution of frequencies. On employing the values of the theoretical ordinates there was found for cell-bodies, mean, 28.5948 micra, standard deviation, 14.8824 micra and coefficient of variation, 18.36 per cent; while for the nuclei, the mean was 13.0535 micra; and standard deviation, 1.7929 micra; the coefficient of variation being 13.73 per cent. When these corrected values are compared with uncorrected ones we find an increase of 3 micra for the mean in both the cells and the nuclei, and a reduction by 10 per cent in the coefficient of variation in the case of the cells and a reduction by 4 per cent in the case of nuclei. These corrected values appear to be the more probable, and are the best we can obtain until some further means of correcting the raw observations have been found.

CLASSIFICATION OF THE SPINAL GANGLION CELLS.

The unavoidable modification in the size of the spinal ganglion cells due to the method of sectioning as here described suggests a revision of the classification of the cells so far as it depends on their observed sizes. It has been mentioned already that using the size of the cells and the arrangement of the stainable masses as criteria, several investigators have attempted to classify the cells composing the spinal ganglion. Three such classifications proposed by LUGARO, LENHOSSÉK and COX will be presented in detail.

LUGARO ('96) distinguishes in the dog five different varieties of the spinal ganglion cells:

1. Large cells with delicate, closely packed stainable masses which are distributed uniformly throughout the cell-body. Around the nucleus are large stainable masses closely packed. The nucleus is large and clear and is provided with a nucleolus. These cells appear to be numerous.

2. Clear, medium-sized cells with irregularly formed small and large stainable masses which are large at the periphery. Even here we see that individual masses are not isolated but are united

together by fine processes. The nucleus is clear and possesses a nucleolus. These cells are most numerous.

3. Small, dark cells with numerous small stainable masses lying in the region of the nucleus. The ground substance becomes diffusely stained. The nucleus also stains diffusely and contains two or more nucleoli. These cells rank third in point of number.

4. Small or medium sized clear cells with large stainable masses which are present in small numbers and connected with each other by processes. The nucleus frequently possesses more than one nucleolus. These cells are not numerous.

5. Large clear cells with long drawn out masses which are continuous with one another and which arrange themselves in concentric lines around the nucleus. These last cells present a laminated appearance like the cross section of an onion. These cells are least numerous.

LENHOSSÉK ('96) in the human spinal ganglion distinguishes three varieties.

1. The first variety consists of cells with a light staining ground substance only. These, which are the largest cells, have a pale ground substance and less numerous, loosely arranged stainable masses, which are most dense around the nucleus.

2. To the second variety belongs coarsely granular cells (grobscholligen Zellen), the appearance of which depends on the arrangement of the stainable substance, and most of the cells in the ganglion belong to this variety. These cells are of medium size, but sometimes small and rarely very large.

3. The third variety contains small cells which have a peculiar internal structure. These cells stain darkly because of the density of the ground substance.

COX ('96) distinguishes in the spinal ganglion of the rabbit two main varieties.

1. One variety contains larger or smaller irregular masses of stainable substance, which do not show a distinct concentric arrangement. The cells of this variety may be either large or small.

2. The other variety contains large, irregular masses of stainable substance arranged concentrically.

It will be clearly seen from the description given by these authors that there exist some structural characters common to both large

and small cells. That is to say, some small cells have characters possessed by large cells and therefore size is the only means of distinguishing two forms. There is however another group of the small cells (third variety of both LUGARO and LENHOSSÉK) which exhibit still different structural characters. They are much darker in appearance owing to a strong affinity for staining reagents. The arrangement of the stainable substance is irregular and indistinct. The cell-outline is irregular. Thus there is no question as to the presence of the two kinds of the small cells which differ in both structure and shape from each other. The entire series of small cells which exhibit a resemblance to the large cells were considered by COX, LENHOSSÉK and LUGARO as early formed cell elements which persist in the spinal ganglion as such in small size. I have however just shown that a considerable number of the large cells are made smaller artificially by the method of sectioning. One would therefore expect to find a number of the small cells similar in structure to the large cells, except that the arrangement of the stainable substance may differ slightly according to plane of section. The cell-outline of the majority of the "artificial" small cells should be nearly spherical, unless they are distorted. Therefore the existence of small cells with the internal characters of the large cells can be explained readily on the assumption that they are in part if not entirely those large cells modified by the method of sectioning. I therefore conclude that a majority of these cells with the characters of the large cells do not preëxist as such and that consequently the conclusions of LUGARO, LENHOSSÉK and COX are to this extent misleading.

While the writer was engaged in the study of the structure of the spinal ganglion in the albino rat (HATAI '01) the following groups of cells were recognized and described. The one group is larger in size and stains lightly with eosin or erythrosin, while another group is smaller in size and stains deeply with eosin or erythrosin. Still a third group which, although it agrees in staining reaction as well as in an irregular outline with the small cells, nevertheless differs in the arrangement of the stainable substance and in size. The size is slightly larger on the average than that of the small deeply staining cells but much smaller than large cells. It now seems better to consider the group intermediate in size as a variety of the small cells rather than as a distinct type. The following are the reasons for this conclusion:

1. The intermediate sized cells agree with small cells in two important characters, the cell-body stains deeply with eosin or erythrosin and the cell-outline is irregular.

2. Since the arrangement of the stainable substance is rather unstable its difference has significance only when other characters also differ from any other given group under consideration. If the recently proposed hypothesis by SCOTT ('05) that the stainable substance is identical with the zymogen granules of the pancreas turns out to be true, then the size and form of the granules as well as their distribution may vary considerably according to the functional condition of the cell.

Consequently we have in the spinal ganglion two forms of cells; one which stains deeply and the cell-outline of which is irregular. Such cells are usually small in size. The other the cell-body of which stains lightly the cell-outline being regular. Such cells range from small to large in size. It must be remembered however that the entire cell-population when they are grouped according to their sizes grade from smallest to largest without showing any interruption. This means of course that there is no definite demarcation line to divide the large from the small cells or vice versa. As a matter of fact, the cells which stain lightly and which also exhibit regular outlines are by no means constant in size. This is also true for the group of the cells which stain deeply and which exhibit an irregular outline, although they are more uniformly small. For this reason, the size of the cell-body is not a proper criterion by which to classify them. The writer has adopted the staining reaction of the cell for classification because, as has been mentioned above, the ganglion cells fall readily into one of the two classes: that is (*a*) those with a deeply stained cell-body with irregular cell-outline and (*b*) lightly stained cell-body with regular cell-outline.

Although we should expect to find intermediate forms, which must always be present, nevertheless grouping by this method is more definite and practical than by the size and is much simpler than by those proposed by other investigators. In addition, these different histological characters are undoubtedly associated with different physiological states (HATAI '01).

I have chosen from NISSL's nomenclature two terms by which to designate the two groups of the ganglion cells with the idea that they may aid description.

1. Pycnomorphic cells, those cells which appear darker owing to a stronger affinity to the staining reagents. The cell-outlines are irregular. Such cells are usually small in size.

2. Apycnomorphic cells, those cells which appear pale owing to weaker affinity for the staining reagents. The cell-outlines are regular, being either spherical or oblong. Such cells range from small to large and include those which are made artificially smaller owing to the method of sectioning.

Attention is called to the fact that the above classification does not modify our views concerning the existence of three histological varieties of cells recognized in the spinal ganglion (see p. 1) but merely shows that these varieties do not distinguish themselves by their diameters in such a way as to form separate groups under this method of examination.

ON THE CORRELATION BETWEEN CELL-BODY AND NUCLEUS.

The intimate physiological relations existing between the cell-body and the nucleus suggest that there may also exist a definite size or mass relation between these two structures. Generally speaking in the growing spinal ganglion cells (HATAI '01), the cell-body grows much faster than the nucleus. It was my object to determine this mass relation, between the cells and nuclei and if possible to find some mathematical expressions by which such relation could be concisely stated.

The correlation table (Table II, p. 490) furnishes us all the data necessary to determine such a relation. The table shows the range of variates in one character corresponding to that in the other. The coefficient of correlation would be then a numerical expression of the occurrence of the several values of x in one character in association with the several values of y in the other. PEARSON gives the formula for obtaining the coefficient of correlation in the following form:

$$r = \frac{\Sigma\,(x.\ y.)}{n.\ \sigma_1\,\sigma_2}$$

Using the above formula, the value of r (coefficient of correlation) was found to be 0.8616 ± 0.0055. This shows that the size of the cell-body is highly as well as positively correlated with the size of the nucleus. Therefore we infer that the larger cell-body is associated with larger nucleus, and vice versa. We can also

find from the correlation table the diameter of the nucleus corresponding to the any given diameter of the cell-body. The value of the nucleus thus obtained is however affected by a variable probable error owing to insufficient number of observations combined with a random sampling. We therefore need to find the most probable values from the observed data, or the characteristic equation which can best represent the data with minimum error. We have two kinds of characteristic equations, linear and non-linear. Whether or not a given expression can be best represented by the linear or non-linear characteristic equation is of the utmost importance, and it is necessary to determine which equation applies to our present data. PEARSON ('04) has introduced a new constant, η, called the correlation ratio and this is used to test the linearity of the regression. The correlation ratio according to PEARSON is the ratio of the variability of the means of the arrays of one correlated character to the total variability of that character and is shown in the following formula:

$$\eta = \frac{\sigma_{my}}{\sigma_y}.$$

The constant η has the same value as the coefficient of correlation when the regression is perfectly linear. If the regression is not linear η will be greater than r. Then evidently $\eta - r$ is a measure of the approach of the regression to linearity. I have calculated the value of η by the formula given above and found that when this value is compared with the coefficient of correlation the former is significantly greater than the latter as is shown in the following:

$$\eta - r = .9267 - .8616 = .0651$$

However, the difference between the value of these two constants will in practice deviate more or less from zero. It is therefore necessary to find whether or not the difference found between the two constants is significant. Recently BLAKEMAN ('05) has given methods of obtaining the probable error of various functions of $\eta - r$. If we let

$$\zeta = \eta^2 - r^2$$

an approximate formula for the probable error of ζ, *i.e.*, E_ζ, is

$$\frac{\zeta}{E_\zeta} = \frac{\sqrt{n}}{0.6745} \cdot \frac{1}{3} \sqrt{\zeta} \frac{1}{\sqrt{1+(1-\eta^2)^2-(1-r^2)^2}}$$

Applying this formula it was found that

$$\zeta = .1164 \pm .0135$$

Thus the difference is certainly significant and data demand a non-linear characteristic equation. I have applied PEARSON'S method of parabola ('04, '05) to the present data and obtained very satisfactory results as will be seen later. The general formula of parabolas of any order is as follows:

$$y = y_0 \left\{ \varepsilon_0 + \varepsilon_1 \left(\frac{x}{l}\right) + \varepsilon_2 \left(\frac{x}{l}\right)^2 + \varepsilon_3 \left(\frac{x}{l}\right)^3 + \cdots\cdots \right\}$$

where l is a half range of variates and ε_s are the constants to be determined from the observed data. I found that for the present

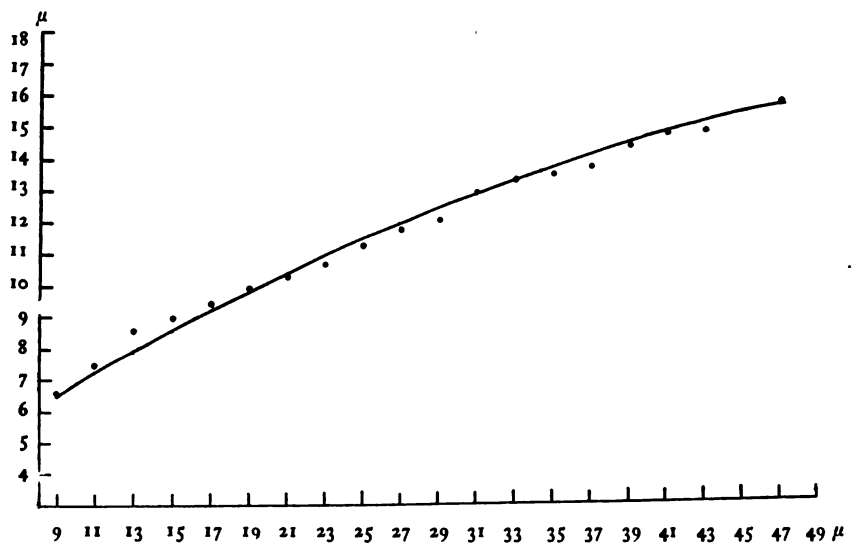

FIG. 4. Probable diameter of the nucleus for given diameter of the cell-body. ..... observed; ————, calculated.

data the parabola of the second order makes a very close fit to the observed means of the arrays. The smooth curve in Fig. 4, where the observed and calculated results are graphically represented, was plotted from the following equation:

$$y = 12.2939 \left\{ 1.0252 + .3564 \left(\frac{x}{l}\right) - .0758 \left(\frac{x}{l}\right)^2 \right\}$$

As will be seen from Fig. 4, the two curves agree very satisfactorily. It is therefore concluded that there certainly exists some definite mass relation between the cell-body and nucleus and its relation is mathematically expressed by the parabolic formula of the second order, as given. Since the regression is not linear but is best represented by parabola we may say that gain in the diameter of the nucleus following increase in the diameter of the cell-body varies in every stage and although the curvature is not pronounced from the nature of the parabola, the diameter of the nucleus is relatively greater in the small cells than in the large cells. For example, when the volume of the cell-body is compared with that of the corresponding nucleus, the following relation is found: In the cell-body whose diameter is 9 micra the volume of the same is 2.64 times that of the nucleus, while in the cell-body whose diameter is 47 micra its volume is 25.34 times that of the corresponding nucleus. This fact indicates, as was mentioned already, a predominant growth of the cell-body over that of the nucleus.

This gives us a method for comparing at some future time the relations in the small cells in the adult ganglion with that of the small cells having the same size in the immature ganglion.

CONCLUSIONS.

We see from the preceding observations that: 1. The method of section modifies the true frequency distributions of the cells and nuclei when their diameters are considered. 2. Under the circumstances the skew curves of Type 1 for the cell-bodies and that of the Type 4 for the nuclei may be considered the best and most reasonable representation of the frequency distribution of the diameters. 3. The theory that the entire group of small cells with the structural characters of the large cells represents unchanged small cells is probably erroneous in view of the unavoidable modification of the large cells by the method of section. 4. The diameters of the nucleus and that of the cell-body are highly and positively correlated ($r = 0.8616$). 5. There exists a definite mass relation between cell-body and nucleus, and the diameter of the nucleus corresponding to any given diameter of the cell-body is best represented by a parabola of the second order. 6. The spinal ganglion cells in a given ganglion may be considered as a homogeneous group, so far as the size is concerned. 7. Spinal

ganglion cells are classified into two groups according to their structural characters: (*a*) *Pycnomorphic cells*, those cells which appear dark owing to a stronger affinity to the staining reagents; and the cell-outline of the same is usually irregular. Such cells are usually small in size. (*b*) *Apycnomorphic cells*, those spherical or oblong cells which stain lightly and have cell-outlines which are regular. Such cells range from small to large in size. These two groups however grade into one another

APPENDIX. TABLE II.

Correlation between cell-bodies and nuclei.

Nuclei / Cell-bodies	4.65–5.30μ	5.30–5.95	5.95–6.60	6.60–7.25	7.25–7.90	7.90–8.55	8.55–9.20	9.20–9.85	9.85–10.50	10.50–11.15	11.15–11.80	11.80–12.45	12.45–13.10	13.10–13.75	13.75–14.40	14.40–15.05	15.05–15.70	15.70–16.35	16.35–17.00	17.00–17.65	
8–10μ	2	1	1	2	2		1														9
10–12		3	2	6	6	4	3	2													26
12–14			2		4	5	8	6	1												26
14–16				1	3	7	14	16	3	1											45
16–18			2			8	12	44	22	2		1									91
18–20						4	3	55	50	20	4	2									138
20–22						6	1	37	76	36	25	11	2		1						195
22–24						1	1	14	58	47	35	17	6	1							180
24–26								7	20	22	25	14	14	4	1	1					108
26–28									8	7	14	26	14	3	2						74
28–30									1		11	10	7	3		1					33
30–32											8	8	10	13	8	5				1	53
32–34										1		5	5	5	11	6					33
34–36											1	2	9	4	9	4	1	2			32
36–38									1			1	3	1	6	6	3				21
38–40											1			1	10	3	2	6			23
40–42														1	2	4	4		2		13
42–44															1	1				1	3
44–46															1	2					3
46–48																	1		1		2
Totals.....	2	4	7	9	15	35	43	181	240	136	124	97	70	36	52	33	11	8	3	2	1108

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From The Wistar Institute of Anatomy, Philadelphia, Pa.

STUDIES ON THE VARIATION AND CORRELATION OF SKULL MEASUREMENTS IN BOTH SEXES OF MATURE ALBINO RATS (MUS NORVEGICUS VAR. ALBUS).

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The present investigation was undertaken to determine the size of the skull and the relative development of its constituent parts in the adult albino rat. To this end the biometric method was employed with the idea that in this way it would be possible to obtain more precise results and also with the idea of later comparing these skulls with those of the hybrids between Mus norvegicus and Mus rattus.

MATERIALS AND METHODS OF MEASUREMENT.

For the present study 53 male and 51 female skulls of mature rats (rats more than 150 days old) were measured. The following measurements were made with vernier calipers: (1) the length of the entire skull; (2) the fronto-occipital length; (3) the zygomatic width; (4) the length of the nasal bone; (5) the height of the skull; (6) the width of the cranium or the squamosal distance. In every case the maximum length alone was recorded.

In the present paper the horizontal straight line joining the tip of the nasal bone to the end of the occipital bone is called the length of the entire skull. This however is not exactly equal to the sum of the length of the nasal bone and that of the fronto-occipital.

The fronto-occipital length was determined in the following way: Since the length measured with the calipers from the tip of the nose to the posterior end of the inter-parietal bone is not always equal to the length measured from the tip of the nose to the end of the occipital bone, both measurements were taken (See Fig. 1). The latter measurement is usually the longer. The difference between the two measurements was added to the length from the tip of the frontal bone to the end of the inter-parietal bone, and the sum was called the fronto-occipital length.

The width of the cranium (squamosal distance) was determined by taking the maximum distance between the two points (right and left) where the zygomatic bones rest on the lateral walls of the cranium. The height of the skull was determined by measuring a perpendicular distance between the greatest convexity of the parietal bone in the median line and the junction line between the basi-occipital and the basi-sphenoidal bones on the ventral surface.

The cranial capacity was determined in the following way: The skull was held vertically, with the nose downwards and was filled with fine shot (No. 11) to the upper level of foramen magnum and then the nose of the skull gently struck twice against the palm of the hand. The space

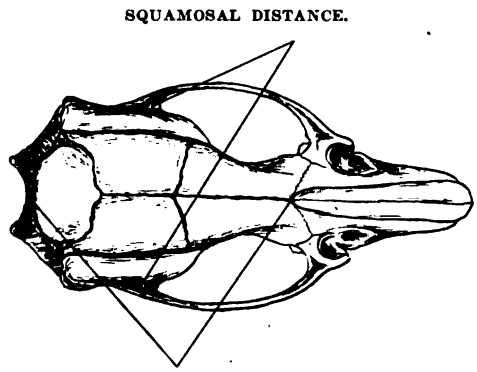

FIG. 1. Diagram of the skull of the adult albino rat, seen from above.

thus formed was again filled. Although this is a simple procedure yet it needs the greatest care and much practice in order to produce uniform results. The slightest variation will easily cause differences of more than one gram in the weight of the shot. The greater the experience of the observer the more uniform are the results. By practice the author has been able to reduce the difference between the first and second filling to less than one decigram in eight cases out of ten. The distribution of the errors in this work was found to follow the Gaussian normal curve and therefore it is inferred that the number of minus errors is the same as the number of plus errors. As a matter of fact the average difference between the first and second fillings did not exceed one per cent. The cranial capacity thus determined was finally transformed into cubic centimeters of brain substance (see page 435).

TABLE I.

		Mean.		Standard deviation.		Coefficient of variation.		No. of Rats.
			Difference.		Difference.		Difference.	
Length of the entire skull.	♂	43.255 ± 0.166	1.706 ± 0.204	1.786 ± 0.117	0.530 ± 0.144	4.129 ± 0.271	1.113 ± 0.338	53
	♀	41.549 ± 0.119	3.944 %	1.256 ± 0.084		3.016 ± 0.202		51
Zygomatic width.	♂	21.745 ± 0.109	0.820 ± 0.137	1.177 ± 0.077	0.301 ± 0.064	5.412 ± 0.356	1.226 ± 0.453	53
	♀	20.925 ± 0.083	3.771 %	0.876 ± 0.059		4.186 ± 0.280		51
Length of the nasal bone.	♂	16.958 ± 0.096	1.266 ± 0.122	1.038 ± 0.068	0.245 ± 0.086	6.121 ± 0.403	1.068 ± 0.526	53
	♀	15.692 ± 0.075	7.465 %	0.793 ± 0.053		5.053 ± 0.338		51
Fronto-occipital length.	♂	27.264 ± 0.093	0.911 ± 0.126	1.007 ± 0.066	0.103 ± 0.090	3.693 ± 0.242	0.256 ± 0.334	53
	♀	26.373 ± 0.085	3.341 %	0.904 ± 0.060		3.427 ± 0.229		51
Squamosal distance.	♂	15.273 ± 0.010	0.217 ± 0.040	0.388 ± 0.022		2.213 ± 0.145		53
	♀	15 056 ± 0.039	1.420 %	0.409 ± 0.027	−0.071 ± 0.036	2.716 ± 0.181	−0.503 ± 0.232	51
Height of skull.	♂	11.493 ± 0.049	0.354 ± 0.065	0.526 ± 0.084	0.151 ± 0.043	4.576 ± 0.300	1.210 ± 0.375	53
	♀	11.139 ± 0.035	3.080 %	0.375 ± 0.025		3.366 ± 0.225		51
Cranial capacity.	♂	10.896 ± 0.068	0.528 ± 0.098	0.735 ± 0.048		6.745 ± 0.444		53
	♀	10.368 ± 0.070	4.845 %	0.743 ± 0.050	−0.008 ± 0.069	7.166 ± 0.481	−0.421 ± 0-655	51
Body-weight.	♂	214.886 ± 5.318	47.541 ± 5.982	52.887 ± 3.760	32.413 ± 4.068	25.076 ± 2.675	12.841 ± 2.847	45
	♀	167.345 ± 2.739	22.170 %	20.474 ± 1.605		12.235 ± 0.974		37
(Length × width × height)$^{\frac{1}{3}}$.	♂	16.875 ± 0.044	0.452 ± 0.056	0.478 ± 0.031	0.096 ± 0.040	2.832 ± 0.186	0.506 ± 0.242	53
	♀	16.423 ± 0.036	2.678 %	0.382 ± 0.026		2.326 ± 0.155		51

Determination of the Means and Variability of the Several Measurements.

In Table I are exhibited the means of the several measurements, the standard deviations, the coefficients of variation and the differences between the two sexes, with their respective probable errors. These values were determined by the usual biometric formulæ (Davenport, 04). As one would expect, the mean values in the males are always higher than in the females. Since in the present investigation the total number of measurements of both sexes was not large, it is important to compare the differences between the sexes with the corresponding probable errors in order to see whether or not the differences here found are to be considered significant. Table I shows clearly that in all cases the differences are greater than three times the probable errors. The maximum difference occurs in the nasal bone; ten times the probable error, and the minimum occurs in the width of the cranium, five times the probable error. This indicates that the characters under consideration are really greater in the male. The maximum percentage differences occurs in the nasal bone (7.5 per cent) and minimum in the width of the cranium (1.4 per cent) while the remaining differences are nearly similar (3.1 per cent to 3.9 per cent).

From the percentage differences here found it would seem probable that if we compared the mature male and female skulls having *the same total length,* the length of the nasal bone in the male would be longer than that in the female, and since the length of the entire skull depends on the combined length of the nasal bone and the fronto-occipital length, it would follow that the fronto-occipital length or the length of the cranium, would be less in the male than in the female. In order to test this conclusion in any individual case it is necessary to determine whether or not the total length of the skull and of the nasal bone on one hand, and the total length of the skull and fronto-occipital length on the other hand, are closely correlated. This point will be more fully discussed after the coefficients of correlation have been determined.

(a) *Range of variates and rate of increase of the various characters associated with changes in the size of the entire skull.*—The two extremes of the various characters, as well as the rate of increase of those characters associated with the increase in the length of the entire skull, is somewhat different according to sex as is shown in Table II.

As is shown in Table II, the absolute range between the two extremes is always slightly greater in male than in female except in the case of the

width of the cranium or squamosal distance and in the fronto-occipital length where they are nearly alike in the two sexes. Generally speaking the changes associated with the increase in the length of the entire skull are relatively greater in female than in male. This is especially evident in the length and width of the cranium and the zygomatic width, although the absolute amount of change is considerably less in the female than in the male. In the female the relative change is very slightly greater in the nasal bone and is the same in the height of the skull. The same table

TABLE II.

	Male.			Female.		
	Minimum mm.	Mean* mm.	Maximum mm.	Maximum mm.	Mean* mm.	Minimum mm.
Length of the entire skull.	39.4	43.3	47.4	44.5	41.5	38.9
Rate	100	100	100.	100.	100.	100.
Zygomatic width.	19.6	21.7	24.8	23.4	20.9	18.9
Rate	49.8	50.2	52.3	52.5	50.3	48.5
Length of the nasal bone.	14.7	17.0	18.7	17.8	15.7	14.4
Rate	37.3	39.2	39.3	40.0	37.7	37.0
Fronto-occipital length.	24.9	27.3	28.8	28.2	26.4	24.9
Rate	63.2	63.0	60.7	63.3	63.5	64.0
Squamosal distance.	14.6	15.3	16.2	16.2	15.1	14.4
Rate	37.0	35.3	34.1	36.4	36.2	37.0
Height of Skull.	10.4	11.5	13.0	12.2	11.1	10.3
Rate	26.4	26.5	27.4	27.4	26.8	26.4

shows us clearly that in respect to the length of the entire skull, the width and length of the cranium are greater in female than in male although in both sexes the absolute amount of change is less than for any other characters measured. The relatively slight increase in the width of the skull (zygomatic width) associated with the increase in the length of the entire skull, has also been noticed by Allen, 94, in the case of Neotoma micropus.

When the mean values (Table II) are treated in the same manner as the two extremes, additional light is thrown on the changes following the increase in the length of the entire skull. In all characters, except the

* Taken from Table I.

length of the nasal bone, the female gives relatively greater values than the male. Although the excess shown in the female is not large in the case of the zyomatic width, and in the case of the height and length of the cranium yet the width of the cranium or squamosal distance is decidedly greater in the female than in the male, as has already been seen from the measurements of the extremes. When mean values for the length of the entire skull are reduced to the same standard and the associated measurements are compared, all the three diameters of the cranium of the female are seen to be relatively greater than those of the male in respect to the length of the entire skull. The cube root of product of these three diameters in the case of the male is 39.01 per cent of the length of the entire skull and in the case of the female 39.52 per cent, thus indicating that we might expect the relative capacity of the female cranium would be sensibly greater than that of the male. This apparent superiority of the female cranium over that of the male is not due, however, to the relatively greater lengths of the three cranial measurements of the female, but is due to the fact that the nasal bone in the male skull is considerably longer, thus producing a somewhat less percentage value for cube root of the product of the three diameters of the male cranium. As a matter of fact, when the length of the entire skull is equated either to male or to female standard by means of the characteristic equations and the resulting measurements of the cranium in the two sexes are compared, the size of the cranium in the two sexes is almost identical. We shall discuss this point later (page 436). It is therefore enough at the present moment to see that the somewhat greater percentage values obtained from the three diameters of the cranium, when mean values for the length of the entire skull are reduced to 100, indicate that the nasal bone is much shorter in the female, and vice versa.

(b) *Relative variability in the two sexes.*—The relative variability in the two sexes is a question which has passed through various phases during the last century. The several opinions held by different investigators are fully summarized by Havelock Ellis in his book on "Man and Woman," 94. The history of this question may therefore be omitted. It is, however, important to note here that the quantitative investigation of this question has been made for the most part on the human subject.

As is shown in Table I, the standard deviation is in every case greater in the male than in the female except in the case of the squamosal distance. Since the standard deviation measures the amount of concentration of the variates about the mean, the greater the standard deviation the less will be the concentration and consequently the greater will be

the variation in the character under consideration. Therefore the greater values obtained for the male means simply that the characters in question vary more in the male than in the female.

The absolute value of the standard deviation is widely different throughout the table. This is due to the fact that in these cases the standard deviation is a concrete number and therefore the variabilities can not be directly compared with one another since the magnitude of the characters as well as the unit taken for grouping is never the same. The coefficient of variation, however, enables us to compare the relative amount of variability of the characters measured in different units since it is one hundred times the quotient of the standard deviation divided by the means. From Table I it was found that the values of the coefficients of variation in the male are always greater than that in the female, except in the case of the squamosal distance in which the reverse is true.[1] Here also the length of the nasal bone shows the greatest variation and the zygomatic width comes next while the least variation is found in the width of the cranium. Brewster, 97, also noticed a greater variation in both the length of the nasal bones and zygomatic width than in any other measurements made on the different parts of the skull. His studies were made on the lynx (Lynx canadensis); cat (Felis domesticus); and red fox (Vulpes fulvis). The methods employed by Brewster for determining his coefficients of variation are so different from those used in the present investigation that two sets of figures can not be directly compared. Except the length of the cranium, the remaining characters tend to show the existence of a sexual difference as to the relative variability, that is the male tends to vary more than in the female.

The mean values obtained from the cranial capacity, body-weight, and cube root of the product of the height, length and width of the cranium are also greater in the male than in the female. The standard deviations as well as the coefficients of variation indicates a relation similar to that found in the skull measurements, that is, the male tends to show in these characters a greater variability than the female.

(c) *Coefficients of variation in man and rat.*—The following table was compiled in order to show the variation in the human skull as compared with that for the skull of the albino rat:

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[1] Slightly greater variability in the female is also found in the cranial capacity, nevertheless the result is insignificant owing to the greater probable errors in this case.

TABLE III.

	Male.	Female.	Investigator.
SKULL CAPACITY.			
Etruscan	9.58	8.54	Pearson and Lee.
Modern Italian	8.34	8.99	Pearson and Lee.
English	8.28	8.68	Macdonell.
Egyptian mummies	8.13	8.29	Pearson and Lee.
Modern German	7.74	8.19	Pearson and Lee.
Naquada	7.72	6.92	Fawcett.
Parisian French	7.36	7.10	Pearson and Lee.
Aino	7.07	6.90	Pearson and Lee.
Albino rat	6.75	7.17	Hatai.
SKULL HEIGHT.			
English	4.21	3.96	Macdonell.
Aino	3.67	3.18	Pearson and Lee.
German	4.47	3.91	Pearson and Lee.
Albino rat	4.58	3.37	Hatai.
SKULL LENGTH.			
English	3.31	3.45	Macdonell.
Naquada	3.09	2.96	Fawcett.
Aino	3.20	3.08	Pearson and Lee.
German	3.37	3.57	Pearson and Lee.
English (base of skull)	4.07	4.11	Macdonell.
Albino rat	3.69	3.43	Hatai.
SKULL BREADTH.			
English	3.75	3.54	Macdonell.
Naquada	3.42	3.42	Fawcett.
Aino	2.76	2.68	Pearson and Lee.
German	3.89	3.39	Pearson and Lee.
Albino rat	2.21	2.72	Hatai.

As will be seen from Table III, so far as the cranial measurements are concerned the coefficients of variation in the albino rat are slightly less than in man, though the difference is by no means large. It is interesting to note that the magnitude of variability both in man and rat is in the same order for corresponding characters, that is the variability of the cranial capacity is considerably greater than the remaining three linear measurements both in man and rat. This is perhaps due to the fact that the cranial capacity is itself highly variable and in addition the technical difficulties of determination of the capacity influence the results further.

Pearson, 97, 01, with reference to variation in the several dimensions of the human skull, thinks that with advance in civilization woman tends to gain in variability on man (see on Aino and Naquada races). Nevertheless, if we examine the data recently obtained by Fawcett, 02,

and Macdonell, 02, Pearson's conclusion, so far as the measurements of the skulls are concerned, is not well supported. For instance the total averages of the coefficients of variation in man (Aino and Naquada races are excluded) are 4.99 per cent while in woman it is 4.84 per cent. Even if we include Aino and Naquada, the relative variability is still in favor of man. The same is true also for the albino rat (4.31 per cent male, and 4.17 per cent female). Therefore so far as the data at hand are concerned the several measurements of the male skulls show a general trend to a greater variability than those of the female. Since in all cases, the number of the skulls examined is not large, it is evident that this point needs still further study.

Determination of the Coefficients of Correlation.

The degree of correlation between any two characters is usually determined by the formula: $r = \frac{\varepsilon(x.y.)}{n.\ \sigma_1\ \sigma_2}$, where x, y are deviations from the means of the two correlated characters and δ_1, δ_2 the respective standard deviations. It will be advantageous to discuss the coefficients of correlation under five headings.

(a) *Correlation between the length of the entire skull and the other cranial measurements.*

TABLE IV.

	Male.	Female.	Difference.
Length of entire skull and zygomatic width	.948 ± .011	.836 ± .029	.113 ± .031
Length of entire skull and fronto-occipital length	.946 ± .010	.956 ± .008	—.010 ± .013
Length of entire skull and length of nasal bone	.845 ± .027	.890 ± .020	.044 ± .034
Length of entire skull and squamosal distance	.582 ± .061	.309 ± .085	.273 ± .105
Length of entire skull and height of skull	.555 ± .064	.314 ± .085	.241 ± .107

As one would expect, the degree of correlation in the first three cases is very high in both sexes. Since the length of the skull depends on the length of the nasal bone and the fronto-occipital length, any change in the length in the entire skull must involve a change in the length of either the nasal bone or the fronto-occipital length or in both. The correlation shows, however, that change in the length of the skull is associated with changes in both the nasal bone and the fronto-occipital length; the latter

change being the better correlated. The high correlation between the length of the skull and the zygomatic width means a regular enlargemen in the transverse diameter associated with a change in length. The correlation between the length of the entire skull and width (squamosal distance) and height is comparatively low in both sexes. Therefore it is concluded that change in both width and height of the cranium corresponding to the change in the length of the entire skull is less regular than in the other three characters already discussed. It is of interest to note that although the correlations between the length of the entire skull and the width and height of the cranium are not high, yet the correlation between the width and height of the cranium is high, especially in the male. (See Table V.)

(b) *Correlation between the height, length, and width of the cranium.* —Table V shows the coefficients of correlation between the three linear measurements just mentioned.

TABLE V.

	Male.	Female.	Difference.
Fronto-occipital length and squamosal distance	.665 ± .052	.397 ± .080	.268 ± .095
Fronto-occipital length and height of skull	.571 ± .063	.387 ± .080	.184 ± .102
Squamosal distance and height of skull	.707 ± .046	.425 ± .077	.282 ± .090

As the table indicates the width (squamosal distance) and height of the cranium give the highest correlation in both male and female, the length and width come next, while length and height give the lowest correlation. Although in every case the male sex gives the higher correlation, the comparison between the differences and probable errors shows that we can not lay much weight on this apparent superiority, except in the case of the height and width, since in the two other cases the differences are smaller than three times the probable error. For comparison there are no data available except the observation by Pearson and Lee, 01, on the human skull, their results are given in Table VI.

TABLE VI.

		Male.	Female.
Length and breadth	German	.286 ± .062	.488 ± .052
	Aino	.432 ± .059	.377 ± .073
Length and height	German	—.098 ± .067	.314 ± .061
	Aino	.501 ± .054	.349 ± .075
Breadth and height	German	.071 ± .067	.276 ± .063
	Aino	.345 ± .064	.178 ± .082

Pearson and Lee's data show that the correlation in the first two instances (length and breadth, and length and height) are about the same and are much higher than that of the last case (breadth and height). In the albino rat, length and width, and length and height also give nearly the same degree of correlation as in man, but the values obtained are smaller than for the width and height. Thus in this respect the rat and man show reverse relation. The difference is probably due to differences in relative development of the several bones of the cranium depending on the skull form characteristic for the two species.

Whether or not it is a general phenomenon in the lower mammals that the coefficient of correlation is higher in the male than in the female, as is shown in the albino rat, needs further observation. It has been pointed out by Pearson, 97, that in the human race with advancing civilization woman tends to gain in correlation on man. It is clearly seen from the data given above that in German skulls the coefficients of correlation tend to be higher in the female than in the male while in the Ainos the reverse relation is true, favoring the view maintained by Pearson. Pearl, 06, who compared a large number of brain records with other body characters in the case of Bohemians, Bavarians, Hessians, and Swedes found that the weight of the brain also tends to be more highly correlated with other characters in the female than in the male.

(c) *Correlation between cranial capacity and other cranial measurements.*—The cranial capacity as determined with the shot gives high degree of correlation with the length of the entire skull as well as with the three diameters of the cranium. This is shown in the following table:

TABLE VII.

	Male.	Female.	Difference.
Cranial capacity and length of the entire skull	.678 ± .050	.484 ± .072	.194 ± .088
Cranial capacity and fronto-occipital length	.761 ± .039	.577 ± .062	.184 ± .073
Cranial capacity and squamosal distance	.838 ± .028	.632 ±.057	.206 ± .063
Cranial capacity and height of skull	.760 ± .039	.666 ± .053	.094 ± .066
Cranial capacity and (height × length × width)⅓	.836 ± .028	.854 ± .026	—.018 ± .038

As is shown in Table VII the coefficients of correlation are in general higher in the male than in the female, but when compared with their respective probable errors the differences are not large enough to warrant laying much stress on the apparent superiority in male sex, except in the one case of the cranial capacity and squamosal distance. It is therefore

safe to say that according to this test the degree of correlation is nearly the same in both sexes. It is interesting to note that the cranial capacity is best correlated with the width (male and female) and the height (male) of the cranium. Therefore a prediction of brain weight based on the cranial measurements would give least error when based on the width. The length of the entire skull gives the lowest degree of correlation in both sexes. This is of course what one would expect since the length of the entire skull is least correlated with both the height and width of the cranium, especially in the case of the female. The product of the height, length, and width of the cranium is highly correlated with the cranial capacity and the correlation, although a trifle higher in the female, is nearly the same in the two sexes. This is also an anticipated result since roughly speaking the capacity should be closely related to the product of three diameters. In this case one might expect to find the correlation almost unity, but remembering that the cranial cavity has an irregular shape and is bounded by curved surfaces, the value shown in the table can be considered satisfactory.

(d) *Correlation between cranial capacity and body-weight.*—Despite the fact that in the human subject the coefficient of correlation between brain and body-weight is extremely low (0.167 in male and 0.226 in female, Pearl, 06,) an intimate relation between these two characters found in the rat (Donaldson) suggests that in the rat at least it would be higher.

As a matter of fact the following coefficients[2] of correlation have been obtained:

TABLE VIII.

	Male.	Female.	Difference.
Body weight and cranial capacity...	.516 ± .074	.692 ± .058	.176 ± .094

Assuming that the regression between the body and brain weights can be expressed by a straight line with a given angle, the following equations were formulated and used to determine the correspondence between the predicted and observed values.

(1) Brain-weight, male = (0.0072 × (body-weight, male) + 9.349) ÷ 5.980.

(2) Brain-weight, female = (0.0251 × (body-weight, female) + 6.168) ÷ 6.009.

[2] Unfortunately in this series, several rats which were found dead are included and thus the correlation here obtained may be slightly less than it should normally be. It is however true that the normal fluctuation in body-weight is rather wide and therefore the including of several self-dead rats should not affect the results to any great extent.

5.980 and 6.009 are two factors for the transformation of the observed weight obtained from shot, into the estimated brain-weight. In other words, if we apply the characteristic equations which enable us to compare the cranial capacity in terms of the weight of shot from the observed body-weight, directly to the adult rats with known body- and brain-weight. (We have a large number of records which give the observed brain-weight accompanied by the corresponding body-weight) then the observed brain-weight was found to be 1/5.98 of the weight of shot in the case of male and 1/6.009 of the weight of shot in the case of female. This simple numerical relation between the observed brain-weight and shot-weight was found to be quite consistent and indeed the characteristic equations with these two new factors have given very satisfactory results in the test thus far.

(e) *Coefficients of correlation in man and rat.*—The coefficients of correlation between the cranial capacity and other cranial measurements in man have been determined by several investigators. The following table shows these relations in man as well as in rat:

TABLE IX.

	Male.	Female.	Difference.	Investigator.
CORRELATION BETWEEN CRANIAL CAPACITY AND LENGTH OF SKULL.				
Aino	.893 ± .016	.663 ± .053	.230 ± .055	Pearson and Lee.
English	.597 ± .051	.691 ± .040	.094 ± .065	Macdonell.
German	.515 ± .050	.687 ± .037	—.172 ± .062	Pearson and Lee.
Naquada	.501 ± .054	.599 ± .039	—.098 ± .067	Fawcett.
Albino rat ...	.761 ± .039	.577 ± .062	.184 ± .073	Hatai.
CORRELATION BETWEEN CRANIAL CAPACITY AND BREADTH OF SKULL.				
Aino	.561 ± .053	.502 ± .070	.059 ± .083	Pearson and Lee.
English	.631 ± .048	.646 ± .044	—.015 ± .065	Macdonell.
German	.672 ± .037	.707 ± .034	—.035 ± .050	Pearson and Lee.
Naquada	.434 ± .058	.532 ± .044	—.098 ± .072	Fawcett.
Albino rat ...	.838 ± .028	.632 ± .057	.206 ± .063	Hatai.
CORRELATION BETWEEN CRANIAL CAPACITY AND HEIGHT OF SKULL.				
Aino	.544 ± .054	.521 ± .068	.023 ± .087	Pearson and Lee.
German	.243 ± .064	.451 ± .054	—.208 ± .084	Pearson and Lee.
Albino rat ...	.760 ± .039	.666 ± .053	.094 ± .066	Hatai.
CORRELATION BETWEEN CRANIAL CAPACITY AND (HEIGHT × BREADTH × LENGTH).				
Aino	.795 ± .039	.780 ± .037	.015 ± .054	Pearson and Lee.
German	.701 ± .034	.814 ± .023	—.113 ± .041	Pearson and Lee.
Naquada	.674 ± .044	.793 ± .025	—.119 ± .051	Pearson and Lee.
Albino rat (height × breadth × length)$^{1/3}$	.836 ± .028	.854 ± .026	.018 ± .038	Hatai.

As is shown in the table, the coefficients of correlation are higher in the rat than in the averages from the human subject in every case, except the cranial capacity and length of skull where the female rat is slightly low. The correlation is decidedly greater in the rat in the case of the capacity as related to the width and height, while in the case of the length and the product of the three diameters the results for the rat are close to those for man. Here again we notice that in the female the coefficients of correlation are slightly greater than in male in the case of the human skulls (except Aino) while in the rat the reverse is true. We can not lay too much stress on this relation, however, since as is shown in the column marked " difference " the size of the probable errors shows the differences to be without significance. Thus although, the general tendency is to show that in the human skull, except Aino, the coefficients of correlation are slightly greater in female nevertheless any definite statement must be postponed until we have data sufficiently abundant to further diminish the value of the probable errors.

(f) *Comparison between the observed and predicted values of skull measurements.*—In Table I we noticed that the mean values of the male characters are always greater than those of the female, the differences always being more than three times the probable errors. The greatest difference was found in the length of nasal bone, the least in the width of the cranium, while the remaining characters gave intermediate values. The question now arises: How the several characters will be related if the length of the entire skull of the male is reduced to that of the female? In other words, is the male skull to be considered as an overgrown female skull, or the female skull an undersized male skull? To answer this question a number of characteristic equations were prepared. These equations will enable us to determine the probable values of the characters in both sexes. The form of the characteristic equation is as follows:

$$Y = \bar{y} + \gamma \frac{\sigma_y}{\sigma_x}(X - \bar{x})$$

where X, Y are the two characters under consideration, $\bar{x}$, $\bar{y}$ are the two respective means of the arrays, σ_x, δ_y are also the two respective standard deviations and γ is the coefficient of correlation. The following table was made in order to show the values of the characters when the lengths of the entire skulls were equated.

As is shown in Table X, when the length of the entire skull of the male is equated to the observed length of the female skull and vice versa, the sexual differences become very small. The closeness of agreement between observed and predicted values of the several characters varies with the

standard taken. This is due to the fact, as Tables IV.-VIII show, that the correlations is higher in male in some cases and in female in others. Five out of seven, characters in the male are absolutely greater than those in female even when the length of the entire skull in two sexes is equated. However the differences are too small to be significant, except in the case of the length of the nasal bone and perhaps the zygomatic width. The nasal bone is significantly longer in the male while the zygomatic width is slightly greater in the female. On the other hand if we equate the

TABLE X.

Probable values of male characters with mean skull length equal to that of the observed female.				Probable values of female characters with mean skull length equal to that of the observed male.			
Characters and No. of equations.	observed ♀	Probable ♂	Difference %	Difference %	Probable ♀	observed ♂	Characters and No. of equations.
Fronto-occipital length. I.	26.37	26.36	.07	—.89	27.51	27.26	Fronto-occipital length. II.
Squamosal distance. III.	15.06	15.09	—.19	.30	15.23	15.27	Squamosal distance. IV.
Height of skull. V.	11.14	11.21	—.67	1.69	11.30	11.49	Height of skull. VI.
Length of nasal bone. VII.	15.69	16.12	—2.64	1.81	16.65	16.96	Length of nasal bone. VIII.
Cranial capacity. IX.	10.37	10.42	—.50	.37	10.86	10.90	Cranial capacity. X.
Zygomatic width. XI.	20.93	20.68	1.17	—.80	21.92	21.75	Zygomatic width. XII.
(Height × length × width)$^{\frac{1}{3}}$. XIII.	16.43	16.43	—.06	.53	16.79	16.88	(Height × length × width)$^{\frac{1}{3}}$. XIV.

length of the female skull to that of the male then the greatest difference is noticed in the length of the nasal bone also and the height of the skull comes next. Besides these two the remaining characters give differences which are always less than 1 per cent. Taking all the results together, we reach to the conclusion that aside from the nasal bone, and perhaps the zygomatic width and height of skull too, the actual sexual differences in the remaining characters are inconsiderable, being less than 1 per cent. This suggests that the nasal bone in the rat may be considered as one of the secondary sexual characters. Consequently the female skull can not be considered as an undersized male skull nor the male skull an overgrown

female skull since these two skulls show at least one significant difference, i. e., in the length of the nasal bone (perhaps zygomatic width also). On the other hand the female cranium, i. e., if we disregard the length of the nasal bone and zygomatic width, may be considered as an undersized male cranium and vice versa, since the differences observed from the three measurements of the cranium in the two sexes are too small to be significant.

According to general belief the female brain is relatively heavier than that of the male although absolutely lighter. Blakeman, 05, found however, that "The Englishman of the same age, stature and diametral product as the mean woman has 1235 grs. brain-weight, or only 10 grs. more than the average woman. The Englishwoman of the same age, stature, and diametral product as the mean man has 1315 grs. brain-weight, or only 13 grs. less than the average man." He concludes from the above that "as far as present evidence goes, we can safely conclude that there is no sensible relative difference in the brain-weights of man and woman, the absolute differences observed are quite compatible with the differences which result from the relative size of the two sexes." The same conclusion, as has been given by Blakeman, may be drawn from the present study on the albino rats. It was found (see Table X) that when the length of the entire skull of the male rat is equated into the length of the entire skull of female, and vice versa, the resulting values for the cranial capacity in the two sexes are almost identical. The difference is in average less than 0.5 per cent, indicating that the sexual difference found in the cranial capacity is entirely accounted for the difference in the size of body. It is also interesting to note in our case that only one character has been equated and therefore if we took a multiple regression-equation the difference would probably almost disappear.

(g) *Characteristic equations.*—I have put together on the opposite page all characteristic equations which have been used in the course of the present study. Equations 1-14 will enable us to find the probable values of the other characters of the skull in two sexes when we know the length of the entire skull, while from the equations 15 and 16 we can obtain the probable brain-weight from the observed body-weight.

The characteristic equations show clearly that the relation between the length of the entire skull and the other characters of the skull, and brain and body-weight can not be determined by simple arithmetical proportion but require in each case the introduction of two or more of the necessary constants which are specific for the character chosen. It follows therefore that in general if the relation existing between the two characters turns

out to be skew (that is a non-linear regression) then the relation should be more complicated and a simple proportion would fail to correctly express the relations existing between the two characters under consideration. On the other hand if the regression is linear the relation may sometimes, but not always be shown by a simple proportion just as well as by a characteristic equation.

(1)	Fronto-occipital length,	♂ = 0.5826	Entire skull length,		♂ + 4.226
(2)	Fronto-occipital length,	♀ = 0.6663	"	"	♀ − 1.811
(3)	Squamosal distance,	♂ = 0.1160	"	"	♂ + 10 515
(4)	Squamosal distance,	♀ = 0.1004	"	"	♀ + 10.884
(5)	Height of skull,	♂ = 0.1634	"	"	♂ + 4.425
(6)	Height of skull,	♀ = 0.0967	"	"	♀ + 7.246
(7)	Length of nasal bone,	♂ = 0.4911	"	"	♂ − 4.285
(8)	Length of nasal bone,	♀ = 0.5619	"	"	♀ − 7.654
(9)	Capacity of cranium,	♂ = 0.2790	"	"	♂ − 1.172
(10)	Capacity of cranium,	♀ = 0.2868	"	"	♀ − 1.527
(11)	Zygomatic width,	♂ = 0.6243	"	"	♂ − 5.259
(12)	Zygomatic width,	♀ = 0.5880	"	"	♀ − 8.298
(13)	(Height × length × width)$^{\frac{1}{3}}$,	♂ = 0.2590	"	"	♂ + 5.672
(14)	(Height × length × width)$^{\frac{1}{3}}$,	♀ = 0.2119	"	"	♀ + 7.619
(15)	Brain-weight,	♂ = (0.0072	Body-weight,		♂ + 9.349) ÷ 5.980
(16)	Brain-weight,	♀ = (0.0251	"	"	♀ + 6.168) ÷ 6.009

Conclusions.

1. All the characters here examined are absolutely greater in the adult male than in the adult female.

2. The coefficients of variation reveal the fact that the male characters show a general trend to a greater degree of variability than those of the female. The length of the nasal bone and the zygomatic width show a much greater variation than any other skull characters in the two sexes.

3. The coefficients of correlation are always positive and tend to be higher in male than in female. The correlation between cranial capacity and body-weight was found to be quite high (0.516 in male and 0.692 in female).

4. The brain-weight corresponding to the observed body-weight may be calculated from the following two characteristic equations:

Brain-weight, male $= (0.0072 \times (\text{body-weight, male}) + 9.349) \div 5.980$.

Brain-weight, female $= (0.0251 \times (\text{body-weight, female}) + 6.168) \div 6.009$.

5. The observed sexual differences are considerably reduced when the length of the entire skull is equated to either the male or female standard. When the lengths of the entire skull in the two sexes are equated and

the remaining characters are compared, the greatest difference is found in the length of the nasal bone (mean differences amount to more than 2 per cent), the height of skull and width of zygoma come next (slightly over 1 per cent), while the smallest differences are found in the remaining characters (less than 1 per cent). From the relation shown above the writer inclines to consider the relative development of the nasal bone in the rat as one of the secondary sexual characters.

6. From the above it is clear that the female skull can not be considered as an undersized male skull, nor the male skull as an overgrown female skull, since there is at least one significant difference in the skulls of the two sexes; i. e., the length of the nasal bone.

7. The female cranium on the other hand may be considered as an undersized male cranium, and vice versa, since the differences found from the three cranial measurements in the two sexes are too small to be significant.

8. The relation between the coefficients of correlation and regression is linear.

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THE CONUS ARTERIOSUS IN TARPON ATLANTICUS (CUVIER & VALENCIENNES)

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THE CONUS ARTERIOSUS IN TARPON ATLANTICUS (CUVIER & VALENCIENNES).

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The tubular prolongation of the arterial end of the heart furnished with numerous valves, and known as the conus arteriosus, is one of the characteristic features of elasmobranchs and ganoids. In *Amia calva*, the conus arteriosus is relatively shorter than in other ganoids, and its valves are reduced to three transversely arranged tiers. It may be said that the absence of the conus arteriosus as a separate structure is a characteristic of the teleostean heart, and that this fact is emphasized by a few recorded exceptions, all of which occur in teleostean families more or less closely related to *Amia*.

Among these exceptional teleosts only one has been hitherto described, which possesses more than one tier of conus valves, this is *Butirinus* (*Albula*) which has two, and to it may now be added *Tarpon atlanticus*.

The heart from which the following description is taken, was sent to me by Mr. Charles H. Townsend, director of the New York Aquarium. It comes from a specimen 5 feet 4 inches in length.[1] I take this opportunity of thanking Mr. Townsend for his courtesy in sending this heart, also an entire *Tarpon*, 4 feet 4 inches long, the heart of which is shown in Fig. 3.

The conus of *Tarpon atlanticus* resembles that of *Amia calva* in form, but differs from it in being proportionately smaller, in having two tiers of valves instead of three, and in appearing to have been driven into the heart towards the apex, so that, instead of projecting freely from the ventricle, as in *Amia*, it is more or less buried in the latter.

In the natural position, the conus of *Tarpon* is a horizontally placed longitudinal tube, elliptical in transverse section. The longest diameter of the ellipse is dorso-ventral, and measures 16

[1] Measurements include caudal fin.

mm., the shortest measures 11 mm., and is transverse. The conus wall is slightly under 2 mm. in thickness. The total length of the conus varies according to the site of measurement, at the mid-dorsal and mid-ventral lines it measures 8 mm., laterally its measurement increases, until at the mid-lateral line on either side it becomes 10 mm.

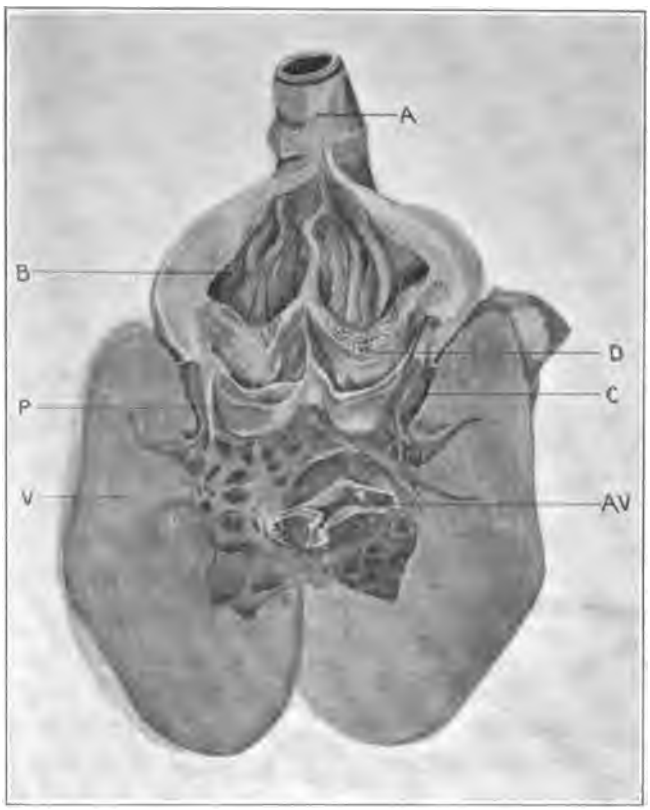

FIG. 1. The heart has been opened by a mid-ventral sagittal incision, and the parts widely separated (natural size). *A*, aorta; *AV*, atrio-ventricular valve; *B*, bulbus arteriosus; *C*, conus arteriosus; *D*, left distal conus valve; *P*, right proximal conus valve; *V*, wall of ventricle.

In order to compare the relative lengths of conus and ventricle in *Tarpon* and *Amia*, the length of the ventricle has been measured by plunging a needle into the apex of the ventricle, in such a direction that its point emerges where the ventricle and conus

blend. In this particular *Tarpon*, the ventricle thus measured is 41 mm. long, and taking 9 mm. as the average length of the conus, the proportion of the conus length to ventricle length, becomes 1 to 4.5. Six *Amia* hearts measured in the same way yield an average proportion of conus to ventricle of 1 to 1.76.

The exterior of the conus presents relations which differ in different regions. At the mid-lateral line, and ventral to this, the ventricle covers the conus completely. Dorsal to the mid-lateral line, the ventricle recedes rapidly, so as only to overlap the conus for a short distance on either side; in the interval, the conus is incompletely covered by the atrium. The area uncovered by ventricle and atrium measured back from the bulbus, is about 3 mm. in the midline, and lateral to this about 4 mm., it is covered by visceral pericardium. (These relations are indicated in Fig. 2.)

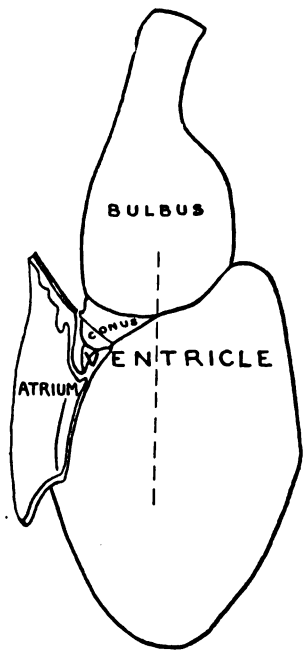

FIG. 2. Diagrammatic right lateral surface-view of bulbus, conus and ventricle. The atrium is represented as incised mesially, and the right half removed (natural size). The line across the conus indicates the site of reflection of visceral pericardium on to the atrium. The broken line indicates the site section in Fig. 3.

The conus is everywhere overlaid by a distinct layer of loose connective tissue, which separates it from the structures which cover it, and renders its outline very distinct in sections of the heart. Owing to the looseness of its connection with neighboring structures, the entire conus is easily exposed from the outside by incising the pericardium at the base of the bulbus, and stripping it away from the adjacent parts of the ventricle and atrium.

The conus valves are disposed in two transverse rows. Each row consists of a right and left cusp symmetrically placed with regard to the median dorso-ventral plane of the conus. Seen rom the lumen of the heart (as in Fig. 1) the valve cusps of

the proximal[1] and distal[1] rows appear to be approximately equal in size, this however is far from being the case, those of the distal row having a capacity far exceeding that of the proximal. The proximal valves are extremely fleshy at their attached margins, and shade rapidly into a thin semilunar area near the free edge; the edge itself is marked by a cord-like thickening, and is quite unattached, except at either end, where, having blended with the corresponding extremity of the other cusp, it is attached dorsally and ventrally to the mid-line of the bulbus a short distance beyond the conus.

The distal valves are not so fleshy as the proximal, and the marginal semilunar area is very thin and profusely perforated. The margins are free except at their extremities, the dorsal ends of the right and left valves blend at the mid-longitudinal region of the bulbus, and become continuous with an elastic cord, the other end of which is attached to the dorsal bulbus wall at its distal extremity. The ventral extremities of the distal valves blend at their point of attachment in the mid-line at the junction of the proximal and middle thirds of the ventral bulbus wall.

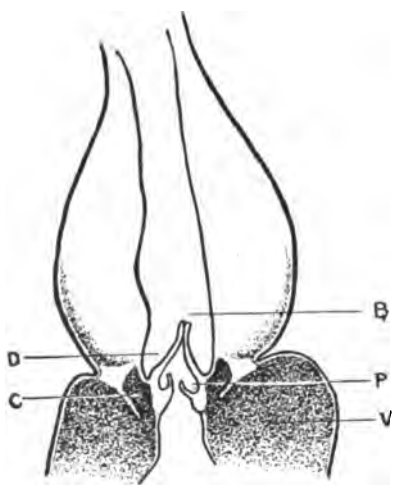

FIG. 3. Ventral face of a frontal section of the heart from the smaller, *Tarpon* its approximate source is indicated by the broken line in Fig. 3 ($\times \frac{1}{3}$). *B*, lumen of bulbus arteriosus; *C*, conus arteriosus; *D*, cavity of right distal conus valve; *P*, cavity of left proximal conus valve; *V*, wall of ventricle.

That the capacity of the distal valves is much greater than that of the proximal, is shown by the fact that a probe can be passed 11 mm. into the former, and only 3 mm. into the latter. To illustrate this point, a frontal section passing approximately through the mid-lateral line of another heart is shown in Fig. 3.

[1] The terms proximal and distal are used with regard to the ventricle.

The conus in *Tarpon* appears to differ from that of *Butirinus* (*Albula*) described by Boas ('80) in that it is less overlapped by the bulbus arteriosus, and more deeply buried in the ventricle, also in that it shows no diminution in length dorsally, as compared to the ventral measurement. The two subsidiary valves between the larger ones of the proximal row in *Albula* do not occur in *Tarpon*.

The sinu-atrial valves are two, with strong tensor muscles. There are four atrio-ventricular valves of which two are of large size, and two somewhat smaller. The hepatic vein, at its junction with the sinus venosus, is of almost cartilaginous rigidity, the size of the orifice is reduced by a thin fold of intima on either side, these almost meet mesially to convert the circular orifice into a vertical slit. The folds of intima appear to have no valvular action.

It is singular that since the appearance of Stannius's paper ('46) *Albula* should have enjoyed the reputation of being the only teleost provided with a conus having two rows of valves; whether the heart of *Megalops cyprinoides* will also prove to have more than one row of valves is an open question. So far as I am aware a description has not been recorded.

Of the other fishes showing evidence of near relationship to *Amia* the following have been examined with a negative result: *Elops saurus* by J. Mueller ('46), *Hyodon* by Mueller ('46) and Boas ('80), *Osteoglossum* by Mueller ('46) and Boas ('80), *Notopterus* by Boas ('80), *Mormyrops* by Mueller ('46). I have also examined *Elops saurus* (for a specimen of which I hereby beg to thank the authorities of the U. S. National Museum) *Hyodon tergisus* and *Notopterus borniensis*.

The original opinion of Gegenbaur ('66) which has been restated and amplified by Hoyer ('00) that the conus, although it has ceased to exist as a separate structure in the ordinary teleost heart, is represented by the portion of the myocardium adjacent to the aortic valves, is well illustrated by the conus relations in *Tarpon*. One has only to imagine the connective tissue layer between the exterior of the conus and the ventricle to have disappeared, allowing the conus muscle to be merged into the general myocardium, and the transition is complete; the relation of the myo-

cardium to the distal valve will be similar to that generally found in teleosts. An interesting transitional stage can be seen in the heart of *Dorosoma cepedianum*, where there is an extremely thin but distinct streak of connective tissue projecting into the myocardium for sufficient distance to clearly separate the areas of original conus and original ventricle.

PHILADELPHIA,
October, 1906.

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NOTE ON THE CONUS ARTERIOSUS OF MEGALOPS CYPRINOIDES (BROUSSONET)

H. D. SENIOR, M.B., F.R.C.S.

[Reprinted from BIOLOGICAL BULLETIN, Vol. XII., No. 6, May, 1907.]

NOTE ON THE CONUS ARTERIOSUS OF MEGALOPS CYPRINOIDES (BROUSSONET).

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Since describing the conus arteriosus in *Tarpon Atlanticus*[1] I have been fortunate in securing a specimen of *Magalops cyprinoides*. For this I take the present opportunity of thanking Professor David Starr Jordan.

The fish in question, preserved in alcohol, measures 19 cm. (including caudal fin) so that the heart is extremely small, and is, on account of its somewhat friable condition, difficult to handle.

The conus is everywhere quite obvious from the exterior. Fig. 1, drawn from the left side, indicates that the general form of

FIG. 1.

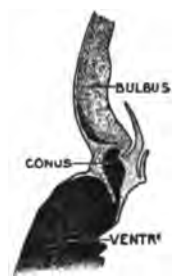

FIG. 2.

FIG. 1. Entire heart of *Megalops cyprinoides* from the left side, × 3. A small portion of the atrium has been removed to display the conus more fully.

FIG. 2. Frontal section through the right side of the heart of *Megalops cyprinoides*, showing the relations in the conus region, × 10.

conus and bulbus resembles that of *Amia* rather than that of *Tarpon*.

The heart was opened by a mid-ventral sagittal incision, it having been previously ascertained that such an incision would fall between the valves without cutting them. After examination and

[1] BIOL. BULL., February, 1907, p. 145. (The literature on the conus arteriosus is given in this article.)

measurement the two halves were imbedded in celloidin and cut into serial sections.

The extreme length of conus is 1 mm. in the mid-ventral line and 1.5 mm. in the mid-dorsal and lateral lines. The ventricle, from apex to junction with conus, has a mean measurement of 5.5 mm. The proportion in mean length of conus to ventricle is therefore 1 to 4.

The conus contains two transverse tiers of valves, each tier consisting of a right and left cusp placed symmetrically with regard to the mid-sagittal plane. The general arrangement agrees closely with that found in *Tarpon*, but the proximal cusps appear to be proportionately more capacious.

The conus in *Megalops* not only projects more freely from the ventricle than in *Tarpon*, but is of greater proportionate length. It would seem to resemble more closely the conus of *Albula* (as described by Boas, '80) except in the absence of the subsidiary valve cusps of the latter.

It should be noticed that the heart described is from a young fish, also that the measurements are, at best, approximate; therefore, comparisons with adults of other genera, if pushed too closely, are apt to be misleading.

The atrio-ventricular valve has three cusps.

A specimen of *Chanos chanos* (Forskål), for which I also have to thank Professor Jordan, presents an easily recognizable vestigial conus arteriosus, but only one tier of valves.

ST. LOUIS, MO., February 1, 1907.

SOME EXPERIMENTS ON THE DEVELOPING EAR VESICLE OF THE TADPOLE WITH RELATION TO EQUILIBRATION

By

GEORGE L. STREETER, M.D.

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From the Anatomical Laboratory of the Johns Hopkins University

SOME EXPERIMENTS ON THE DEVELOPING EAR VESICLE OF THE TADPOLE WITH RELATION TO EQUILIBRATION[1]

BY

GEORGE L. STREETER, M.D.

Associate, Wistar Institute of Anatomy

WITH TWELVE FIGURES

The eventual object of the experiments reported in the following paper was the rearing of some tadpoles which had been deprived of their auditory vesicle and acoustic ganglion, either on one side alone or on both sides; that is to say, an artificial production of a unilateral and bilateral absence of the acoustic apparatus. This was done in the expectation that it might be possible to trace the central acoustic path, in this new way, and perhaps throw further light upon its course and relations. The absence of these sense organs, however, produced such definite abnormalities in the behavior of the growing larvæ and in the development of their swimming abilities that it became at once apparent that I was dealing with valuable evidence in respect to their function and its bearing on the mechanism of equilibrium. It is, therefore, deemed advisable to restrict the following paper to the physiological features of these experiments, and reserve the study of the central nervous system of the reared specimens for a later communication.

What we already know concerning the function of the vertebrate ear is based principally on experimental sectioning or stimulation of the semicircular canals, or the nerves to their ampullæ, in adult birds and fishes.[2]

[1] Read in part before the Section of Anatomy of the British Medical Association, at the meeting held in Toronto, August 21-25, 1906.

[2] For experimental work on fishes we are for the most part indebted to Lee ('93 and '98) and Lyon ('00), both of whom carried on their experiments at the Woods Hole Laboratories. Further work on fishes has just been completed at the same place by Professor Parker, whose paper I am told is now in press and will appear in the Bulletin of the U. S. Fisheries Bureau. An abstract of part of his work was read before the American Zoölogical Society (Parker, '05). A voluminous literature exists concerning experiments on higher vertebrates, particularly the pigeon, but it need not be considered here.

The fact that it is possible to experiment on the embryo and to produce at will practically a congenital absence of this organ, besides serving as a control over the experiments on adult animals, introduces a direct advantage both as regards the ease with which the operation is performed and as regards its completeness and permanence and freedom from injury of adjoining structures, the latter point being of particular importance to those who are still in doubt as to how much is due in the experiments on adults to injuries and stimulations associated with the operation and how much is purely the result of the cessation of the stimuli which normally originate in the labyrinth. Furthermore, since the labyrinth is removed during the early formative period at a time when it may be presumed that the various organs possess their greatest adaptability, it will be seen that such embryonic interference affords a most complete test of the power of functional compensation on the part of other organs.

Behavior of Normal Tadpoles

In analyzing the behavior of operated specimens it was found necessary to make a preliminary study of control tadpoles, in order to determine the normal development of motor reflexes and their coördination and the consequent establishment of equilibrium. This was done by removing the larvæ from their gelatinous capsule shortly after fertilization and following their development in tap water. In this way it was seen that in the process of learning to swim they pass through three periods, which may be named as follows:

1. Stage of non-motility, first three days.
2. Stage of spinal reflexes, fourth to sixth days.
3. Stage of equilibrium, sixth day to maturity.

The first stage, with a favorable temperature, lasts from the time of fertilization to the third or fourth day. During this time the larvæ, aside from the movement due to cilia, lie motionless on their side on the bottom of the dish and do not respond to stimuli. The second stage begins at the time when they first respond to

mechanical stimuli by flexion of the body and tail.[1] These reactions consist of simple motor reflexes at first, but they soon become combined and coördinated so that by a series of such body flexions they are able to wiggle rapidly forward on the bottom of the dish. This manner of progression evidently consists entirely of spinal cord reflexes and is not controlled by higher centers. In order to perform it, it is necessary for the tadpoles to touch the bottom or

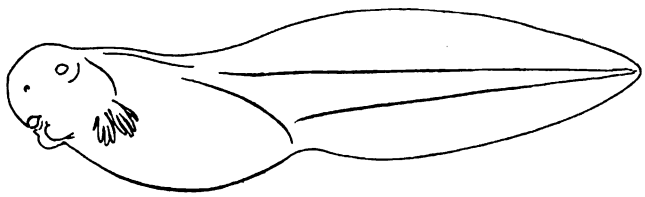

FIG. 1

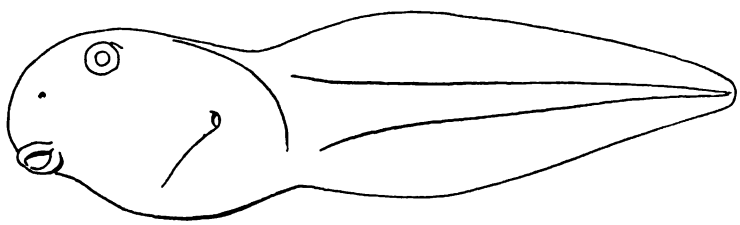

FIG. 2

Fig. 1. Outline drawing of normal tadpole (Rana sylvatica) of the second stage or stage of spinal reflexes. Enlarged 8 diameters.

Fig. 2. Outline drawing of normal tadpole (Rana sylvatica) at the beginning of the third stage. This specimen had the power of equilibration, although sections of the ear vesicle showed that the development of the semicircular canals was not yet complete. Enlarged 8 diameters.

side of the dish; when they are driven up into the free water with a pipette, where there is no contact with solid objects, they make no effort at movement, but sink inertly to the bottom; on striking the bottom they run forward again. The third stage begins when they are first able to move freely about without touching solid

[1] I have been informed by Dr. R. G. Harrison that it is just at this time that the motor nerve roots make their appearance, and this may determine the onset of the second stage. According to his observations the power of muscle contraction follows almost immediately after the development of the motor roots; but it never precedes their development, as is maintained by some. He has found the motor root present in specimens that had not yet moved.

objects. At this time a new control over their movements is developed, in virtue of which they become able to leave the bottom of the dish and swim up into free water with maintenance of what may then be called equilibrium. The form of the tadpole during the latter part of the first stage is shown in Fig. 3. The second and third stages are shown in Figs. 1 and 2.

The correlation between the histological development of the labyrinth and the development of the power of equilibrium was studied by selecting specimens of the second and the beginning of the third stages, carefully noting their behavior, and then cutting them in serial sections.[1]

From these series it could be seen that shortly before the animal enters the stage of equilibrium the labyrinth consists of a closed epithelial sac incompletely subdivided into compartments and possessing differentiated nerve endings which are connected with the brain by the acoustic nerve and ganglion. That at least one such apparatus is essential for equilibrium will be seen when I describe the behavior of tadpoles that have been completely deprived of the same. As regards the semicircular canals it is a different matter; they can already be seen in the process of development, but are not completely pocketed off until after equilibrium is already established. Consequently the semicircular canals as such are not an essential factor in equilibration.

Method of Operation

Larvæ of Rana sylvatica measuring about 3 mm. long were selected as being most suitable for the operation. Their general form at this time is shown in Fig. 3. There is a distinct tail bud, and on the head the eminences caused by the optic cup and head ganglia are visible. The structure that is to form the future labyrinth is situated just dorsal to the ganglionic eminence and is shown

[1] The correlation between the histogenesis of organs and the development of their functional activity forms a fruitful field which has been explored by comparatively few investigators. It may be approached both through ontogeny and phylogeny. Prentiss ('01) by this means worked out important facts regarding the crustacean otocyst. Many details concerning the vertebrate ear which do not belong to the scope of the present paper could doubtless be learned in the same way.

in Fig. 3 by the mark +. It consists of a cup-shaped mass of cells (auditory cup) which have differentiated themselves from the deeper layer of epidermis, and are just in the process of closing in at the edges to form the completed ear vesicle. In size this ear cup or ear vesicle is about one-half that of the optic cup.

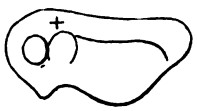

Fig. 3. Outline drawing of Rana sylvatica at the time suitable for operation, just at the end of the non-motile stage. The tail bud is present and on the head are seen the eminences due to the optic cup and head ganglia. Above the latter is the point of operation shown by a cross. Enlarged 8 diameters.

For performing the operation it is not necessary to anesthetize the specimen as it is still in the non-motile stage and does not respond to stimulation. After removing the larva from its gelatinous capsule it is placed under a binocular microscope and an incision made near the place indicated in Fig. 3. The edge of the incision is then raised a little and the auditory cup is picked out with a needle. After a little practice one learns to make the incision directly at the edge of the cup so that it comes away easily and intact, resembling somewhat a thimbleberry. Lying just in front of it is the acoustic ganglion which is not so sharply outlined. This is also removed and, in order to make sure that it is all taken out, the surrounding mesoderm is cleaned out as far in as the brain. Where but one vesicle is to be removed the operation is then complete, and the specimen is left to proceed in its development. The wound immediately closes of itself and heals in the course of a few hours leaving no trace of the operation. Where both sides are operated on, the same procedure is carried out on both sides. The ear vesicle never regenerates following complete removal.

The ear vesicle was removed on one side from thirty specimens and on both sides from twenty specimens. The animals were then kept under observation and their behavior recorded through the whole larval period and until the completion of metamorphosis. The following notes were selected from these records.

Removal of One Ear Vesicle

Twenty-four hours after operation: Specimens are 5.5 mm. long and show presence of gill buds. In appearance and behavior, no difference can be detected between them and normal tadpoles. They lie on their side and on stimulation flex their body, but make no attempt at swimming.

Forty-eight hours after operation: Specimens are 7 mm. long, gills are branched and the blood can be seen circulating through them. In appearance and behavior they still show no departure from that seen in normal control specimens. While at rest they lie on their side. On stimulation (sunlight, jarring the dish, or touching with needle) by a rapid flexion of the body and tail from side to side they swim forward, 5–10 cm., on the bottom of the dish in a straight or slightly curved line, and then come to rest on their side, and remain so until a new stimulation excites another such excursion. Their course is directed either by the side or bottom of the dish. When forced up into free water the flexions stop and they sink inertly to the bottom.

Third day after operation: Specimens average about 8 mm. long, abdominal epidermis differentiated from that of the dorsal parts of the body by being less pigmented. Appearance and behavior is still practically normal. They begin to show a tendency to assume the upright position while at rest, but no great importance can be attached to this feature as throughout the early days of the tadpole period, preserved specimens lie in the same positions as living ones. Their posture in water may be entirely determined by their body proportions. Their movements remain of the spinal cord type seen on the previous day, the response being more prompt.

Fourth day after operation: Specimens 9–9.5 mm. long. In appearance the operated specimens are the same as the normal ones, but in behavior they present a difference. The normal ones still confine their movements to the bottom or side of the dish; when stirred up into free water, though most of them still roll about inertly, some of them are able to maintain a direct course. On the other hand the operated ones, as soon as they are driven from the bottom, swim in a spiral or circular manner as shown in the

accompanying Fig. 4. The tendency is to swim with the operated side under, and in the rolling movements around the long axis of the body it is from the operated side under to the opposite. When these same specimens touch the bottom they are able to direct their course as on the previous two days. Evidently, a functional union is normally established at about this time between the ear vesicle and the spinal cord reflex centers, upon which the individual is dependent for maintaining its position in free water, and it is not until this occurs that the removal of the ear vesicle causes any symptoms.

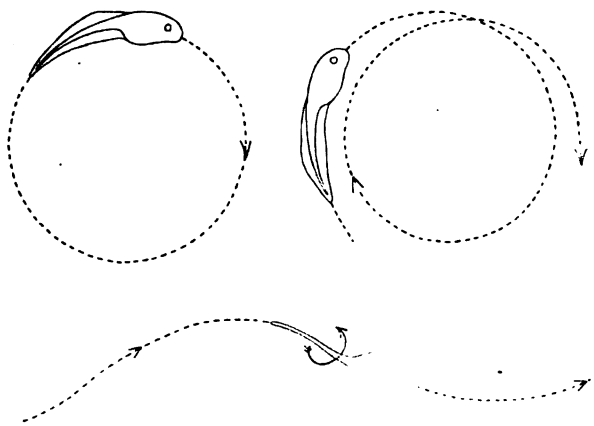

Fig. 4. Sketch showing three typical swimming movements made by specimens on the fourth day after removal of their left ear vesicle.

Sixth day after operation: Specimens about 12 mm. long, and have commenced to nibble at food and pass fæces. The characteristic movements which first appeared on the fourth day have become stronger and stand out in more marked contrast to the behavior of normal specimens which at this time can swim easily up into free water with accurate maintenance of equilibrium.

Seventh day after operation: The operated specimens show distinct improvement in swimming ability; many of them are now able to maintain a fairly direct course in free water, but on excitation they renew the spirals and circles which characterized the fourth, fifth and sixth days.

Eighth day after operation: nearly all the specimens now swim freely and directly in all parts of the water, and irregularity of swimming is only elicited by excitement.

Tenth day after operation: Swimming is practically normal. Their movements are under such control as to enable them to support themselves in free water and nibble at floating stems and leaves. It can be seen, however, that in swimming they lean

Fig. 5. Photograph of a frog whose left ear vesicle was removed when a tadpole 3 mm. long. The only asymmetry noticeable is the absence of the ear elevation on the left side normally caused by the labyrinth and its cartilaginous capsule; the lateral line on that side is straight from the eye back, while on the right or normal side it is deflected. The posture is normal. Enlarged 3½ diameters.

slightly toward the operated side, a sympton which persists throughout their larval period.

Twelfth day after operation: Specimens are normal as regards size, nourishment, and symmetry, except for the absence on the operated side of the elevation which is caused normally by the labyrinth and its cartilaginous capsule. In behavior they differ from the normal only in the slight leaning toward the operated side

and a momentary loss of equilibrium which can be elicited by excitement.

Three months after operation: The specimens passed through a normal metamorphosis at the end of the third month. A photograph made of one of them a few days after the completion of the process is shown in the accompanying Fig. 5.

As long as they continued as swimming tadpoles the slight leaning toward the operated side persisted and it was possible through excitement to cause a momentary disturbance in equilibrium, but the latter became gradually more difficult to demonstrate. As soon as they commenced to make use of their legs the character of the swimming changed; it then became a series of leg strokes instead of the sinuous flexions of the body and tail. After that it was no longer possible to detect the leaning toward the operated side; both when swimming and when at rest their behavior was to all appearance normal. When taken out of water they jumped normally and came to rest in a normal posture. When turned over on their backs they righted themselves promptly.

The fact that the slight disturbance of equilibrium, which could be still detected in the tail-swimming tadpole, could no longer be seen in the leg-swimming frog, a change completed within four or five days, probably does not signify the cure of the condition, but rather that under the latter circumstances a slight defect is more difficult to recognize. The corollary of this would be that equilibrium in the swimming tadpole is a more delicately balanced mechanism than in the kicking and jumping frog.

Removal of Both Ear Vesicles

During the first three days after the operation the appearance and behavior of these specimens are the same as seen in the normal ones, and in those from which one ear vesicle was removed. The response to stimuli is perhaps a trifle less prompt, but otherwise they could not be distinguished one from the other.

Fourth day after operation: It was seen that in one-sided operations the specimens commenced about this time to make excur-

sions into free water, and in doing it they departed from the normal by swimming in spirals and circles. Tadpoles with both ear vesicles taken out make no such excursions and show decidedly less activity. Occasionally they flex their body and tail from side to side producing a snapping effect which does not result in any forward progress. Like the other specimens they are, however, able to wiggle along in contact with the side and bottom of the dish.

Seventh day after operation: The specimens are smaller and are retarded in development as compared with the normal and one-sided specimens. They are, however, symmetrical in form and are normal as regards the appearance and movements of the eyes, mouth, heart and intestine. They are decidedly less active and stimuli produce irregular attempts at swimming, sometimes somewhat spiral in character but usually nothing more than a series of awkward flexions of the body. These flexions also occasionally occur with no apparent stimulus. They make a partially successful effort at nibbling on the bottom of the dish.

Twelfth day after operation: Absolutely no improvement in swimming; any attempt at it results in a series of somersaults. they throw their body up into the water and then promptly sink to the bottom in almost any position. When at rest, they lie on their side, back, or normally on their belly, depending apparently on whether their intestine is filled with sand, etc., to properly balance the body. The intestine is very apt to be empty because of the difficulty they experience in feeding. They do not wiggle along on the bottom as well as they did on the fourth and fifth days.

Two months after operation: The specimens could not be carried much beyond this point, the difficulty apparently being starvation from inability to wander around and collect food. Perhaps also the respiration was involved, for they were unable to go to the surface for oxygen as the normal tadpole does.

In behavior they show no improvement. For the most part they lie stiff and inert in various positions on the bottom, and their occasional attempts at swimming have never developed into anything more successful than was described on the seventh and twelfth

days after operation. Their appearance departs from the normal principally in the small contracted character of the abdominal region. In volume they are about one-third as large as the normal specimen, varying from 2.5 to 4 cm. in length. They have a hind leg bud 2.5 to 3 mm. long. As some of them commenced to die at this time the rest were put in preserving fluids for microscopial purposes.

A *summary* of the above notes on the operated individuals may perhaps be best formulated by making the following comparison with the three stages of normal behavior.

First stage: The operation was performed during the latter part, while the animals were still non-motile.

Second stage: During this period they behave exactly like normal specimens, both those having one vesicle removed and those that have been deprived of both vesicles. They respond to stimuli and learn to wiggle along in contact with the bottom of the dish in the normal manner.

Third stage: It is at the beginning of this period that they depart from the normal. It can be plainly seen from their conduct that something has happened to that controlling influence from above, which they require in order to leave the bottom and to swim and maintain their position in free water. In case but one ear vesicle is gone they swim in spirals, circles, or straight while rolling around their long axis. This, however, lasts only a few days and then it is gradually overcome. From then on they swim almost perfectly; there may be a slight tilting toward the operated side and on excitement a momentary loss of equilibrium, but this would only be seen on careful examination. It is a different matter where both labyrinths are absent; the animals in that case are completely and permanently incapacitated for swimming. There is no apparent sense of equilibrium and they never develop any. The animals were kept alive about two months, at the end of which time their movements were as irregular as at the beginning.

Transplantation of Ear Vesicle After Bilateral Removal

From the above experiments it became evident that a tadpole having but one labyrinth proceeds in its general growth and

develops swimming abilities about as well as the normal animal; but specimens deprived of both ear vesicles never learn to swim and never develop any sense of equilibrium. The next step was to see if it would be possible to remove both vesicles and at the same time transplant one of them into a new position, having in mind the successful results obtained by Lewis ('04) in transplantation of the optic cup.

After that operation if the tadpole succeeded in developing equilibrium and the power of swimming then it would prove that a transplanted ear vesicle could establish new connections with the central nervous system and develop its normal functions; the ship would simply be sailing with its compass set up in a different place.

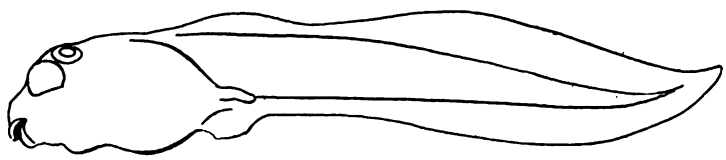

Fig. 6. Tadpole showing elevation in front of eye caused by the transplanted left ear vesicle, the right ear vesicle having been entirely removed. Drawing made three months after operation. Enlarged 4½ diameters.

The operation was one that could be performed without difficulty. A tadpole about 3 mm. long is selected and the ear vesicle taken out on one side in the manner described above. The specimen is then turned over and the opposite ear vesicle is uncovered and loosened from the epidermis. Before actually removing it a straight incision is made with scissors or needles in front of the eye and a pocket is created by gently spreading the subjacent mesoderm apart until the brain is exposed. The loosened ear vesicle is then lifted from its natural place and slipped into this pocket. If the incision is carefully made the edges of the wound close at once and on the following day there is no trace of the operation left. Nine operations of this kind were made and seven of the tadpoles successfully reared. While they were growing it could be seen from a surface view that the transplanted vesicle was developing and causing a corresponding elevation in

front of the eye. A sketch of one of these at the end of the third month is shown in Fig. 6.

Their behavior during the first week following the operation was identical with that of specimens deprived of both vesicles as was to be expected. Toward the fifth and sixth days they could make progress while touching the side or bottom of the dish, but any attempt at swimming in free water resulted only in irregular flexions of the body and somersaults. It was hoped that the transplanted vesicle might then begin to function and make it possible for them to perceive their position while in free water, but this did not occur. They continued to behave in all respects like tadpoles having no labyrinth and never gave evidence of possessing any trace of equilibrium.

At the end of the third, fourth and twelfth weeks specimens were killed in preserving fluid and prepared in serial sections. Examination of the sections showed that in six out of the seven specimens which were cut, the transplanted vesicle had developed to a greater or less extent, and it was these vesicles that formed the surface elevations that had been macroscopically visible in front of the tadpole's eyes. Graphic reconstructions of them are represented in Figs. 7 to 12. It will be seen that none of these constitute a perfect labyrinth, but on closer study it is found that they all possess certain features which are characteristic of it. In the first place, that which was transplanted in the form of an open auditory cup developed after the operation into a closed vesicle containing endolymph. This did not then remain a simple vesicle, but exhibited the tendency to subdivision into two or more compartments, the utriculus and sacculus, as seen in Figs. 7, 8, 12. In the walls of these compartments there are areas of specialized epithelium representing the maculæ acusticæ. In Fig. 7 there opens out of the more dorsal compartment a distinct endolymphatic appendage. A typical semicircular canal is not present in any of them; but what may be called a canal tendency is seen in Fig. 8, where there is a tube uniting the two principal compartments. The small blind pouches leading off the main vestibule, three of which are present in Fig. 10, doubtless represent abortive canals. In transverse section they are perfectly round and look like typical

canals. It may be recalled that Rüdinger ('88) described the semicircular canals as developing in the form of blind tubes sprouting out from the general vesicle. It is quite possible that he was dealing with an abnormal embryo and had the same form of canals that we see in Fig. 10.

The ear vesicles are more or less completely enveloped in connective tissue membranes and they are partly incorporated in masses of cartilage, some of which belongs to the normal cartilaginous cranium and some of it is the regular cartilaginous capsule of the labyrinth, the two fusing together in some places.

In four cases (Figs. 7, 8, 9 and 10) a group of ganglion cells and nerve fibers are attached to the median side of the vesicle near its caudal end and extend toward the central nervous system. In one instance (Fig. 7) the nervous connection between vesicle and brain at the junction of olfactory lobe and fore-brain, is complete, though it is only a few fibers that actually enter the brain. As the acoustic ganglion at the time of the operation is attached to the auditory cup some of its ganglion cells are undoubtedly carried along with it, and it is probable that it is these cells that furnished the nerve connections just described. At the time the transplanted ear cup was slipped into its pocket the adherent ganglion cells must have been lodged in various positions as regards the ear cup and the fact that they all come finally to lie on the median side of the vesicle and lead toward the brain must be explained by some theory of an attraction existing between brain and nerve.

When we have to deal with a transplanted labyrinth that has reached a development equal to those that function in young tadpoles, and has established communication with the central nervous system, we might expect that it would show some sign of physiological activity. The failure of it to do so is perhaps best accounted for by the fact that the point of entrance into the brain is so far away from the hind-brain centers and the spinal cord that connections with these are not established. If the experiments were varied and the vesicle transplanted to some point in the neighborhood of the occipital nerves this difficulty would be obviated.

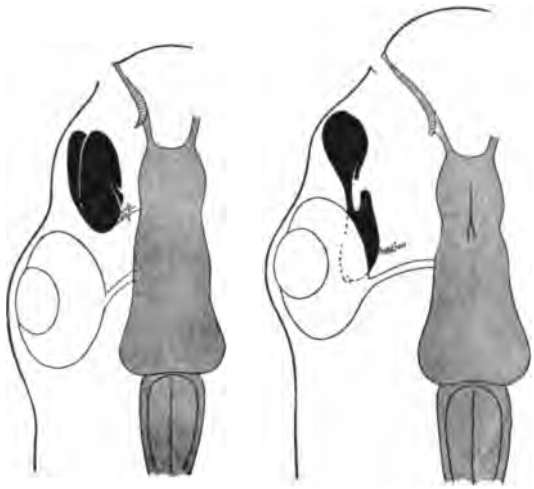

Fig. 7 Fig. 8 Fig. 9

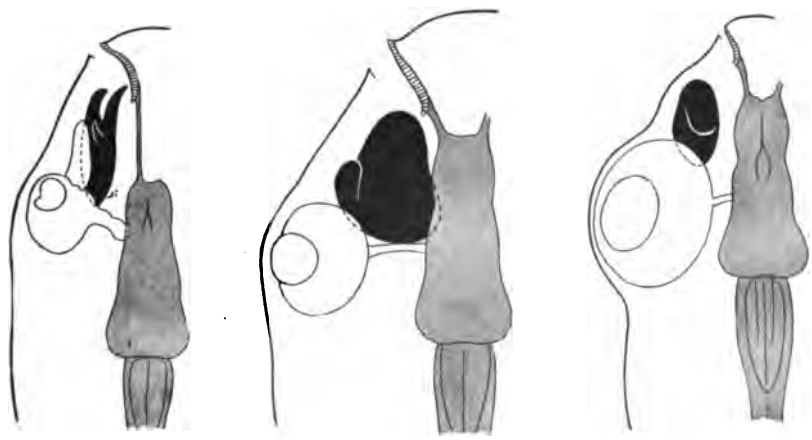

Fig. 10 Fig. 11 Fig. 12

Figs. 7 to 12. Graphic reconstructions showing the form and relations developed by transplanted ear vesicles, one to three months after the operation. In all six cases the right ear vesicle was removed and the left vesicle transplanted into a subdermal pocket between eye and nostril. In Figs. 7, 8, 9 and 10 the acoustic nerve and ganglion extended from ear vesicle toward brain; in Fig. 7 the connection was complete, the fibers entering at junction of fore-brain and olfactory lobe. Central nervous system, shaded; ear vesicle, solid black.

Conclusions

In the tadpole the ear vesicles are essential for the development of the power of equilibration, but the study of normal specimens shows that well developed equilibration may be present before the completion of the semicircular canals; the latter as such are therefore not essential.

When both vesicles are removed no other organ compensates for their loss and the animal is completely and permanently helpless as regards the maintenance of equilibrium. When only one ear vesicle is taken out the remaining vesicle is capable of performing the work of both so perfectly, that the casual observer would mistake them for normal individuals.

Transplantation of the ear vesicle shows that the group of cells forming the auditory cup or primitive ear vesicle is specialized to that degree that although removed from their natural relations and placed in a new environment they still continue to differentiate themselves into a structure approximating the normal labyrinth. A nerve and ganglion develops, and complete nervous connection may be established between the transplanted vesicle and the brain at an abnormal place. Where the latter occurred it did not give evidence of any functional ability.

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A RECENT TENDENCY IN DESCRIPTIVE NEUROLOGY

BY

GEORGE L. STREETER, M. D.

Reprint from THE ANATOMICAL RECORD of THE AMERICAN JOURNAL OF ANATOMY, VOL. VI, No. 3

January 1, 1907

A RECENT TENDENCY IN DESCRIPTIVE NEUROLOGY.

It has been the custom in describing and teaching the central nervous system to divide the brain and cord into the gross morphological units—cerebrum, basal ganglia, thalamus, mid-brain, pons, cerebellum, medulla oblongata and spinal cord with its four regions and two enlargements. These divisions are studied in detail, and it is shown how the various masses are derived from a simple neural tube and its three "primary" vesicles. Extraordinary emphasis is laid on the mechanics of the mysterious chorid plexus and the foramen of Monroe. Having completed the identification of such macroscopic features it is customary to determine microscopically the internal structure of these separate divisions, proceeding from the cord toward the brain or in the opposite direction. Descriptions then usually conclude with the enumeration and course of the projection fibers and paths connecting the morphological divisions. In other words we are accustomed to descriptions of the nervous system based essentially on form, and we visualize it as a piece of inert architecture.

Inasmuch as our first knowledge of the central nervous system pertained to its form it is natural that the first descriptions should take on a morphological character—both in the human brain, toward which attention originally was almost exclusively directed, and also in the case of the comparative and embryological studies which have appeared later. As a consequence we have at our disposal an "orderly" arranged mass of strictly morphological details that may be said to have reached a high degree of completeness. The little that has in the meantime been discovered by other workers concerning the functional significance of the various brain parts has until recently had but small influence on our analysis of the nervous system. The priority and predominating bulk of the morphological matter have completely controlled the custom of descriptive treatment.

The first break in the traditional treatment was about ten years ago when Edinger published his enlarged book of lectures.[1] He had discovered the advantage of combining the study of form and function, and produced a work best characterized as an embryological and comparative anatomy illuminated by experimental and clinical data. Though he does not succeed in entirely breaking away from the time-honored method of dividing the brain, yet he does succeed in making one constantly feel that the brain is a living mechanism. The immediate acceptance of this book and its consequent wide circulation and numerous editions testify to the appreciation of the innovation on the part of his readers.

It was the same feeling for the functional character of the nervous system that influenced Meyer in his paper on brain structure.[2] He is more radical than Edinger, and discards the morphological divisions such as the pons, mid-brain, etc., as units of description. In place he proposes to divide the nervous system into a series of functional transverse laminæ or segments. These do not necessarily correspond to metameres. In the spinal region the portion of cord which corresponds to a single nerve root constitutes a segment. The cranial region he divides into five segments, based on their peripheral connections:

1. Visceral segment, regulating the mechanism of respiration, of articulation and deglutition.

2. Auditory-facial-abducens segment, regulating equilibrium, hearing, and the movement of eye, face, and ear muscles.

3. Mastication segment, regulating movements of jaw and sensation of face and fauces.

4. Optic segment, with optic nerve and muscles of eye-ball.

5. Olfactory segment, the only segment that has afferent fibers alone.

These divisions are shown in the accompanying figure, which is based on Meyer's Fig. 6, the cerebral and cerebellar mechanisms having been omitted.

In these functional segmental units Meyer sees a repetition of type which he resolves into the following elements:

1. Segmental neurones—efferent and afferent nerves.

[1] Edinger, L., Vorlesungen über den Bau der nervösen Zentralorgane des Menschen und der Tiere, Leipzig, 1896. This was preceded a few years by a smaller book of ten lectures for physicians.

[2] Meyer, A., Critical review of the data and general methods and deductions of modern neurology, Jour. of Comp. Neurol., Vol. VIII, 1898.

2. Intersegmental neurones—means of coordinating various segments, *e. g.*, ground bundles.

3. Supersegmental neurones—elaborated centers for special mechanisms, *e. g.*, cerebellum.

With this framework he sketches out the nervous system of low forms, such as the worm, and then proceeds to higher forms, working in, in a general way, the details of the most highly organized nervous system. His proposed analysis possesses many features that commend it for clinical and didactical use.

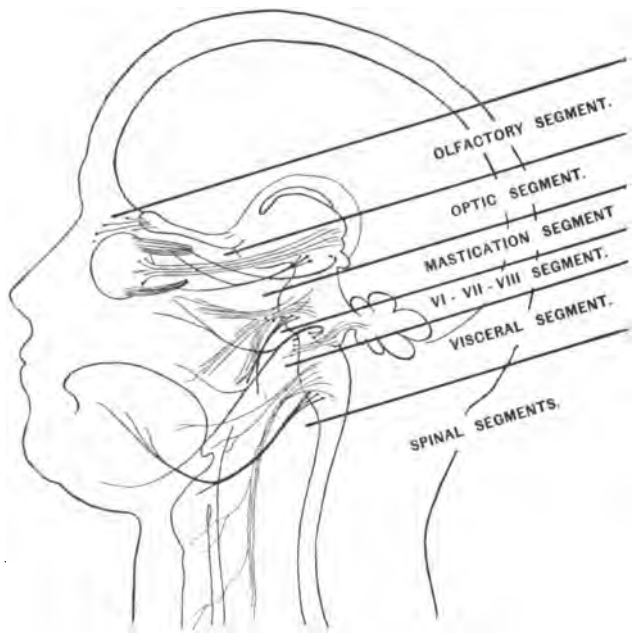

FIG. 1. Diagrammatic profile of the head, showing the subdivisions of the central nervous system proposed by Meyer. Sensory fibers are indicated by fine lines, and motor fibers by heavy lines. The term "segment" is not used in a metameric sense.

A third notable stride toward a functional description of the central nervous system has just been made by Johnston, in a book which is likely to receive much consideration.[3] Like Meyer, he discards the customary morphological method of treatment and traces its structure and phylogenetic history entirely on the basis of function. His functional

[3] Johnston, J. B., The nervous system of vertebrates. Philadelphia, 1906.

units, however, differ from those of Meyer in being made in a longitudinal direction, while those of the latter consist as we have seen of transverse segments.

There are, according to Johnston, two main activities in the vertebrate organism: first, actions in relation to external world (somatic), and secondly, internal activities having to do with processes of nutrition and reproduction (visceral). In each case there is a two-fold activity on the part of the nervous system; reception of stimuli and motor responses. Thus the nervous system consists of four functional divisions:

Somatic sensory elements.
Somatic motor elements.
Visceral sensory elements.
Visceral motor elements.

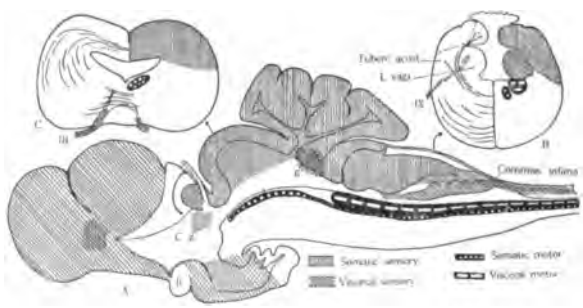

FIG. 2. **Profile and two transverse sections of a simple vertebrate brain, showing arrangement of the four primary divisions of the nervous system. After Johnston.**

With certain exceptions each of these divisions is represented in each segment of the nervous system, and all of the segments of a given division are serially homologous. Structurally and functionally they constitute bilaterally four longitudinal columns, which, according to him, are the primary elements of the nervous system and are more fundamental than the metamerism of the body. The arrangement of these divisions is shown in Fig. 2, which is taken from Johnston's book.

In addition to the four primary divisions there are the group of brain centers and tracts which perform functions of correlation and also the specialized sympathetic system. Under these headings then he classifies the whole nervous mechanism. The method of treatment adopted

by him is an outgrowth in part of his previous studies and in part is based on the work of others, among whom are Strong, Herrick, C. J., and especially Gaskell, who deserves credit for first showing the identity of the visceral and somatic divisions. This, however, is the first time that the whole vertebrate nervous system has been gone over and functionally divided and described in longitudinal systems as has been done in the book under discussion.

Though it is probable that Meyer's system of segments could be readily adapted to didatic purposes by those working with the human brain and would be of immediate advantage clinically; yet the serial overlapping of structures makes any system based on transverse laminæ difficult to apply for purposes of finer analysis. Meyer encounters this difficulty in his facial-abducens-auditory segment. The facial nerve and its pars intermedius belong functionally and structurally to the glossopharyngeus and vagus group, and aside from position, have nothing in common with the vestibulo-cochlear apparatus, with which, however, in his system he is forced to include them. This difficulty is avoided in Johnston's scheme. His longitudinal divisions seem to be completely adequate in the cord and hind-brain. There is much, however, in the fore-brain that such a system leaves involved in difficulties, and in some of his interpretations other people may disagree with him. Thus, regarding the olfactory apparatus, which he classes under the visceral sensory system, there are those who may be inclined to consider it, like the optic apparatus, as belonging to the cutaneous or somatic sensory system. It may be expected that such complexities will be straightened out as we lessen the amount of functional ignorance that still persists concerning this region. Judging from Johnston's success thus far in demonstrating one fundamental (functional and structural) character of the four primary longitudinal systems, we have reason to hope that that there is here an adequate basis for the analysis of the central nervous system.

The work of the writers that have been referred to in this paper indicates that there is a wide-spread feeling of dissatisfaction with the existing cumbersome and lifeless descriptions in neurology; and though it is not safe to predict what the details of the future analysis are to be, it is, nevertheless, evident that the said analysis will be made in terms expressing function.

George L. Streeter.

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THE CORTEX OF THE BRAIN IN THE HUMAN EMBRYO DURING THE FOURTH MONTH WITH SPECIAL REFERENCE TO THE SO-CALLED "PAPILLÆ OF RETZIUS"

BY

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WITH 8 FIGURES

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THE CORTEX OF THE BRAIN IN THE HUMAN EMBRYO DURING THE FOURTH MONTH WITH SPECIAL REFERENCE TO THE SO-CALLED "PAPILLÆ OF RETZIUS."

BY

GEORGE L. STREETER, M. D.,
Associate Professor of Neurology at the Wistar Institute.

WITH 6 FIGURES.

The observations reported in the following paper conclusively show that the cortical granulations, or papillæ of Retzius, caused by the fungiform arrangement of the cells of the pyramidal layer, and commonly found in human embryos between 11 and 14 cm. long, constitute an abnormal condition, which is produced either by intrauterine or postpartum maceration. It is pointed out that of two human brains of the same age one may have cortical papillæ while in the other they may be absent. Furthermore, it is shown in pig brains, where cortical papillæ are not normally present, that it is possible by experimental methods to produce a fungiform clumping of the cortical cells that exactly duplicates the condition seen in human brains.

Attention was first called by Retzius, **95**, p. 17, to the fact that human brains, usually of the fourth month and more rarely of the fifth month, possess a fine granulated character perceptible through the smooth surface of the cortex, and in places where the thin superficial layer (Randschicht of His) had been torn off they appear correspondingly granulated or covered with rounded elevations. Microscopical examination of sections of these regions revealed the fact that the granulated character was due to an unequal growth of the pyramidal cell layer, which projected in rounded elevations, the spaces between which were filled in by the superficial or molecular layer, so that the surface of the brain remained smooth. Retzius considered the possibility of this granulation formation being a manifestation of some pathological process, such as is commonly associated with abortion, *e. g.*, syphilis. He was, however, more inclined to believe it a normal condition due to a transitory exuberant growth of the pyramidal cell layer, the surface irregularities caused thereby being

smoothed out later by the development of the adjacent layers. Shortly after this Hochstetter, **98**, p. 5, briefly refers to the granulations of the pyramidal cell layer described by Retzius. He confirms their presence in poorly preserved brains, and designates this appearance as a decomposition phenomenon, without giving any further evidence.

Two years later His, **00**, not having noticed the observations of Retzius, described independently the same peculiar granular or wart-like character of the pyramidal layer in embryo brains of the fourth month; and again in his last work His, **04**, he describes at some length this appearance under the title "Die Retziusschen Wärzchen." One of his illustrations is reproduced in Fig. 1. Though in his discussion he admits that it is

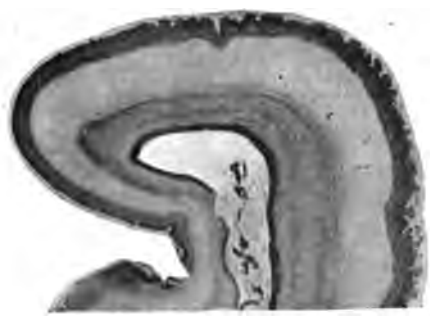

FIG. 1.

FIG. 2.

FIG. 1. Section through the occipital lobe of the brain of a human embryo, 12 cm. long (end of 4th month), showing the irregularities of the pyramidal zone caused by the fungiform clumping of the cortical cells, the so-called papillæ of Retzius. Taken from His, 04, Fig. 75.

FIG. 2. Section from the brain of a human embryo of about the same age and taken from the same place as shown in Fig. 1. Here the pyramidal zone consists of a compact layer with parallel borders, which, with the exception of three transitory fissures presents a perfectly smooth outer surface, and shows no trace of the Retzius papillæ.

still an open question; yet he is apparently inclined to consider the papillæ as normal, and does not hesitate to discard the possibility referred to by Retzius of their being pathological, on the ground that several of his specimens, which showed characteristic cortical papillæ, came from healthy individuals who had committed suicide, and the fœtuses themselves appeared normal. He also argues that if it were a post-mortem alteration, associated with the swelling of the tissues, then the superficial layer would also present an irregular surface, which is not the case.

Neither His nor Retzius accounted for the fact that this phenomenon

has not been observed in brains of other animals. With this in mind the writer decided to make the test on some other mammal, fresh embryos of which could be easily obtained at all ages, and where control experiments could be carried out on embryos of the same litter. The pig was selected, and an examination was made of brains of embryo pigs measuring from 10 to 14 cm. long, which is a period that corresponds to and fully covers the time of appearance of cortical papillæ in the human brain.[1]

It was found that the pyramidal layer in the pig does not exhibit any cortical papillæ when carefully preserved; but has always a smooth, regular surface, the only indentations being those corresponding to the beginning fissures, which make their appearance in specimens between 12 and 13 cm. long. A photograph of a section of a normal brain of a 11.5 cm. pig is shown in Fig. 3.

Having found that cortical papillæ are not normally present in the pig, the next step was to see whether they could not be produced artificially, and preferably under conditions which might be probable in case of human material. Two possibilities suggested themselves as etiological factors; in the first place, maceration of the specimen before it was put into the preserving fluid, and secondly imperfect penetration of the preserving fluid. Under maceration we should have to consider both postpartum and intrauterine maceration. The latter might be brought about, for instance, by disease of, or abnormal attachment of the placenta with consequent disturbance of circulation, and perhaps death of the fœtus some days before abortion. The second condition, faulty penetration of the fixative, might be present in brains of this size were the fixative not injected through the arteries or the brain coverings not immediately opened up so as to permit the direct action of the fluid on the brain itself.

The following experiments were carried out with the idea of imitating these two conditions; on the one hand, for obtaining imperfect penetration

[1] The fact that the cortical papillæ are usually limited to the fourth month may perhaps be explained as follows: Up to that time the brain wall is relatively thin and uniform in structure, so that deformities then take the form of complete foldings of the wall. In specimens of the fourth month the wall is sufficiently thick to prevent foldings of the entire wall, and expansion and shrinkage express themselves in a readjustment of its constituent parts, some parts being more affected than others. In older specimens such a readjustment is prevented by the development of the cell processes and the supporting framework of neuroglia, resulting in a structure sufficiently firm to preserve its form in the fixative, and consequently no more papillæ or artificial fissures are found.

of the fixing fluid, the brain coverings were not removed until the specimen was ready for embedding, and on the other hand, the maceration was produced either by keeping the specimens dry and exposed to the air long enough for them to macerate in their own fluids before they came into the fixative, or in other cases by putting the brains directly into normal salt solution for varying lengths of time. In human material His found the cortical papillæ most marked in material hardened first in formalin and then immersed for several days in Müller's solution. So the same method of fixation was adopted in the experiments, the details of which are as follows:

A. Maceration Followed by Imperfect Fixation.

A1. Maceration in own fluids (11, 12, and 14 cm. pigs).
Embryos left exposed to air, 18 hours.
Embryos placed in formalin, 10%, 48 hours.
Embryos placed in Müller's solution, 4 days.
Washed, brought into alcohol, and then the brain coverings were removed and the brain imbedded and cut in paraffin.

A2. Maceration in normal salt solution (12 cm. pigs).
Embryos placed in salt solution, 17 and 48 hours.
Embryos placed in formalin, 10%, 48 hours.
Embryos placed in Müller's solution, 4 days.
Washed, brought into alcohol, and brain removed as above and the brain then sectioned in celloidin and paraffin.

In these specimens, in which the brain coverings were left intact throughout the period of fixation, no cortical papillæ were found. Embryos of different sizes were tried (A1) for the purpose of covering the whole period favorable to the formation of the papillæ. The poor preservation of the tissues manifested itself by a varying degree of fragmentation of the sections, particularly of the deeper parts. The sections presented a shredded appearance which varied from minute forking clefts between small clumps of cells and between fiber bundles, up to large irregular cracks splitting the different layers of the brain wall. This condition was found both in material that was cut in paraffin and in that cut in celloidin, but it was more marked in specimens that had been macerated in salt solution 17 hours, and still more marked in those macerated 48 hours. Otherwise the general topography of the sections and the arrangement of the layers was fairly well preserved. The minute clefts between the cells of the pyramidal layer gave a slightly ragged appearance to the surface

of that layer, but it was nothing that approched the fungiform arrangement seen in the Retzius papillæ. As can be seen in normal specimens at this time, the pyramidal layer is split by a line of scanty nuclei into a

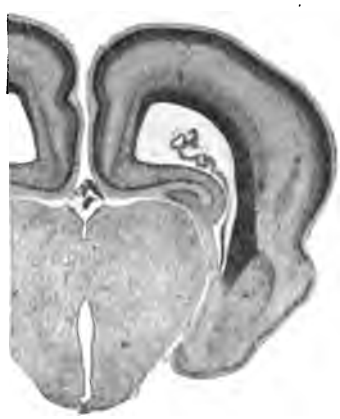

FIG. 3. Section from a well-preserved brain of a pig embryo. 11.5 cm. long. This section shows that normally, in the pig brain of this age, the pyramidal zone presents a uniformly smooth outer surface. This brain, while still warm, was preserved in a chrome-acetic mixture.

FIG. 4. Section from a macerated brain of a pig embryo, 11.5 cm. long. The brain was kept in normal salt solution 48 hours and then preserved in formalin followed by Müller's solution. The section shows distinct fungiform clumping of the cortical cells and characteristic Retzius papillæ. The same specimen is shown under higher power in Fig. 6.

more superficial thicker subdivision, the pyramidal cells proper, and a deeper subdivision which is to form the layer of polygonal cells. This stratification was preserved in the experimental material. Another feature of importance was the absence of the so-called transitory fissures.

B. Maceration Followed by Good Fixation.

B1. Maceration in normal salt solution (11.5 cm. pigs)

(a) Fresh brain placed in salt solution, 28 hours.
Hardened in chrome-acetic solution, 48 hours.
Washed, dehydrated and sectioned in celloidin.

(b) (Figs. 4 and 6) Fresh brain placed in salt solution, 48 hours.
Hardened in formalin 10%, 24 hours.
Secondary fixation in Müller's solution, 4 days.
Washed, dehydrated and sectioned in celloidin.

B2. Maceration in its own fluids (11.5 cm. pig) see Fig. 5.
Embryo left exposed to air, 48 hours.
Brain removed and kept in formalin, 48 hours.
Secondary fixation in Müller's solution, 4 days.
Washed, dehydrated and sectioned in celloidin.

The sections of the specimens macerated in salt solution (B1, a and b) show fairly good preservation of the deeper lying parts, there is almost no shredding of the tissue like that seen in the specimens in which the penetration of the fixing fluids was hindered by the brain coverings. The pyramidal layer of the cortex, however, is found to be thrown into irregular folds, accompanied by a fungiform clumping of its constituent cells. This appearance is present in both specimens, but is more marked in the specimen (*b*) macerated 48 hours. A section of this was photographed and is reproduced in Fig. 6. The resemblance is close to the description given by His and Retzius of the cortical papillæ in the human embryo. It has the same smooth-surfaced superficial layer, which dips down between the papillæ of the subjacent pyramidal layer. In some places these incisures cut off small irregular islands of pyramidal cells. The inner surface of the pyramidal layer does not have these sharp notches, but runs across the section in an irregular wavy ill-defined line. In addition to the fungiform clumping of the cortical cells, in some of the sections the so-called transitory fissures are found. These dip sharply inward and invade in some cases more than one-third of the thickness of the brain wall. In the formation of these, the superficial layer is partially folded in with a corresponding cleft on the surface of the brain, which is not the case with the cortical papillæ. It may be noted that the artificial character of transitory fissures has been well established by Hochstetter, **98**, and Mall, **03**, the latter having examined over fifty embryos and found that according to the effect of various dissociating influences he could obtain macera-

tion in all stages, from simple folding of the brain wall up to conversion of the entire central nervous system into a pulpy mass. Evidently cortical papillæ and transitory fissures, though differing in character, have a similar etiology; as in the above experiment we have both, artificially produced in the same brain under known conditions. The interesting fact should be noted that though the two formations may occur in the same brain, and may closely adjoin each other, yet they do not occur together at the same place; that is to say, one does not find a fungiform grouping

FIG. 5.

FIG. 6.

FIG. 5. Section of the brain of a pig embryo, 11.5 cm. long. The embryo was left exposed to the air, and the brain allowed to macerate in its own fluid 48 hours. The brain was then removed and preserved in formalin followed by Müller's solution. In this section the fungiform clumping involves only the outer part of the pyramidal zone, and in this respect closely resembles the condition seen in Fig. 1.

FIG. 6. Section of same specimen shown in Fig. 4. The maceration here is more advanced than that seen in Fig. 5. The fungiform clumping involves the whole thickness of the pyramidal zone, both the inner and outer surfaces of which are thrown into coarse irregular folds.

of the cells that lie in the cleft of a transitory fissure. Either process seems sufficient to satisfy the space demand.

Sections from the specimen macerated in its own fluids (B2), see Fig. 5, differ from those macerated directly in salt solution in that the fungiform arrangement of the cortical cells involves only the more superficial part of the pyramidal layer. Instead of foldings of the whole layer, such as is seen in Fig. 6, we have here only a granulated or fungiform surface; and this duplicates almost exactly the condition found in the embryo Pl

of His, which he pictures in Figs. 75, 77, 98 and 99. It shows that it is possible by different methods of maceration to produce experimentally typical cortical papillæ in brains where they are not normally present.

Conclusion.

The comparison of Figs. 1 and 2, one with, and one without cortical papillæ, suggests the probability of the abnormal character of the papillæ. One could still perhaps raise the objection that they may be normal, but very transitory, and that the two sections do not quite represent the same stage of development, so that in Fig. 2 the papillæ have either already disappeared or have not yet developed. This objection, however, can no longer be considered in face of the fact that in pigs, where one is able to secure specimens in exactly the same stage of development, it is possible, as has been shown above, to produce the papillæ by means of maceration, and furthermore to control their size and character by varying the degree and method of maceration.

From the experience derived from the above experiments, as regards conditions which predispose to artificial fissures of the cortex and deformities of its constituent cell layers, it becomes evident that embryo brains, which are intended for general morphological study, should, up until the time of completion of the principal fissures, be hardened *in situ* without disturbing the brain coverings. If the brain of a human embryo fresh from the uterus is uncovered or completely removed, and then immediately immersed in formalin or other fixative, it will not necessarily be free from abnormal fissures, etc. The framework of the brain wall up to that time is by no means firm, and it must be also remembered that it may already have been softened by maceration in the uterus. Thus in such a case, and much more so in the embryos that do not reach the hardening fluid so promptly, it is essential that the brain coverings should be left intact, that they may serve as a support to the brain during the process of fixation. Imperfect penetration of the preserving fluid is to be obviated by injecting it through the blood-vascular system.

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SOME FACTORS IN THE DEVELOPMENT OF THE AMPHIBIAN EAR VESICLE AND FURTHER EXPERIMENTS ON EQUILIBRATION

By

GEORGE L. STREETER, M D.

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SOME FACTORS IN THE DEVELOPMENT OF THE AMPHIBIAN EAR VESICLE AND FURTHER EXPERIMENTS ON EQUILIBRATION

BY

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WITH SIX FIGURES

In a previous paper concerning experiments on the developing ear vesicle[1] it was shown that the group of cells forming the primitive epithelial ear cup or ear vesicle of the tadpole is specialized to that degree that although removed to an abnormal environment the cells still continue to differentiate themselves into a structure possessing many of the features of a normal labyrinth. Recently it has been shown by Lewis[2] that even earlier, while still an uninvaginated plate, the ear anlage is already capable of a certain degree of independent differentiation. In the following paper additional evidence will be given of the high degree of developmental independence possessed by the early labyrinth cells. It will be pointed out that individual parts of the vesicle may develop independently of the rest of the vesicle. It will also be shown that the process of differentiation extends to the difference existing between a right and left-sided organ. A left ear vesicle transplanted into the empty pocket left by the removal of the right ear vesicle develops into a labyrinth that is perfect in general form and in its relations to the brain, with the exception that it maintains its left-sided character; the anterior semicircular canal is found on the caudal side toward the vagus group, while the posterior canal lies toward the eye, and likewise the lagena which

[1] Streeter, G. L., '06: Some experiments on the developing ear vesicle of the tadpole with relation to equilibration. Jour. of Experimental Zoöl., vol. iii.

[2] Lewis, W. H., '07: On the origin and differentiation of the otic vesicle in amphibian embryos. Anatomical Record, No. 6, Amer. Jour. of Anat., vol. vii.

normally buds out from the caudal border of the saccule in these cases is found extending forward toward the proötic ganglion.

The ear vesicle, however, is not in all respects independent of the surrounding structures. Some experiments which are reported below, indicate that its position in reference to the brain, ganglion masses and the surface of the body is determined by the environment itself; it may be rotated in any direction, and nevertheless it eventually develops in the normal attitude, with the saccule toward the ventral surface, the semicircular canals toward the dorsal surface, the lateral semicircular canal being toward the lateral surface, and the endolymphatic appendage toward the brain.

The experiments were carried out on larvæ of Rana sylvatica and Rana pipiens, and the operating stage was the same that was used in previous experiments.[3] The time is just at the close of the non-motile stage, and the epithelial ear consists of an invaginated cup-shaped mass of cells just in the process of being pinched off from the deeper layer of the skin, with the edges turning in to form a closed vesicle. For simplicity the term "ear vesicle" will be used even though the closure is not yet complete; the attempt to distinguish between auditory cup and auditory vesicle does not seem to be justified for the present purposes. The technique of the operations was also the same as that described in the previous paper. Notes were made on the behavior of the animals, and at the end of from four to six weeks the specimens were preserved in a chrome-acetic mixture, cut in serial sections, and stained with hæmatoxylin and congo red. With certain specimens the ear vesicle, adjacent ganglia, and a portion of the central nervous system were reconstructed after the Born wax plate method. Eleven such models were made, and photographs of some of them are reproduced in Figs. 2, 3 and 6. With the aid of these models it was possible to identify relations and detailed features of the labyrinths that otherwise could not have been recognized.

The morphological features of the experiments will be first considered, and the behavior of the animals and its relation to equilibrium will be treated separately in the latter part of the paper.

[3] Streeter '06: *l. c.*, Fig. 3, p. 547.

DETERMINATION OF POSITION OF THE EAR VESICLE

The conclusion that the attitude of the developed labyrinth, the position of its canals and various chambers, is determined by its environment is based on seventeen experiments in which the ear vesicle was loosened from its normal situation and placed in an abnormal attitude, and the specimen then allowed to continue in its development. At the end of a month examination showed that the labyrinth had become differentiated with varying degrees of completeness, and in each instance had developed in normal relation to the surrounding structures.

Rotation in Two Directions. In eight of these experiments the ear vesicle was rotated 180° around both its vertical and transverse axes, so that it was turned face inward and upside down; or, in other words, its lateral or invaginated surface was toward the brain and its ventral border was where the dorsal border should be, the maximum displacement. After this procedure the wounds healed within a few hours, and the larvæ were reared up to the fourth or fifth week, when they were killed and cut in serial sections. The labyrinths of five specimens were reconstructed. Before describing them reference should be made to the normal condition of the labyrinth at this age. A reconstruction of a normal one with its adjacent structures is shown in Fig. 1.

From the reconstruction of a normal specimen it can be seen that the three semicircular canals have individual characteristics by which they can be separately identified; such as the Y-shaped union of the anterior and lateral canals, and the overlapping of the caudal end of the lateral canal by the posterior canal, and the junction of the posterior and anterior canals to form the crus commune. The differentiation between utricle and saccule is not yet complete, but the part that is to become saccule is so labeled. From the caudal border of the saccule can be seen a small pocket budding out which constitutes the lagena or primitive cochlea. Directly median to the crus commune is the endolymphatic appendage, consisting of a small duct leading from the main labyrinth chamber up between the labyrinth and brain to a rounded pouch, the saccus endolymphaticus. In their histology, as well as in

their general form, the various parts of the labyrinth exhibit at this time individuality. See Fig. 4. The ventro-median portion of the vestibular sac and the ampullar ends of the semicircular canals possess high columnar cells forming the neuro-epithelial maculæ which are supplied with fibers from the acoustic ganglion, lying against the medial wall of the labyrinth. The endolymphatic sac has cuboidal cells, and the lagena has intensely staining columnar cells like those seen in the macular regions. The lagena is further characterized by its sharply rounded outline, and by the fact of its being compactly surrounded by ganglion cells and fibers, and cartilage forming cells. These features are so definite that

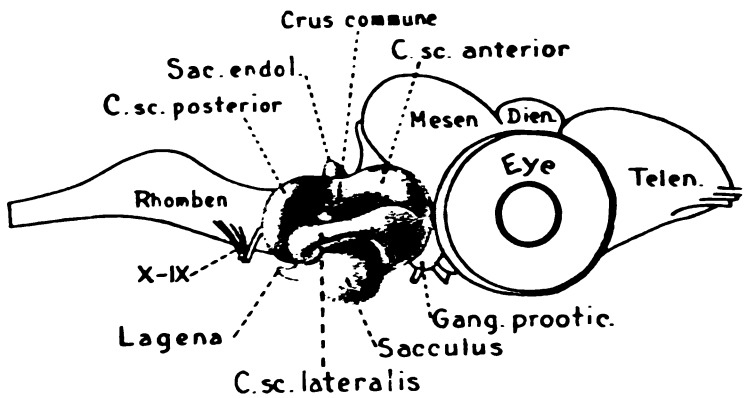

Fig. 1 **Reconstruction showing the form and relations of the membranous labyrinth of a normal** tadpole (Rana pipiens) one month old. The labyrinth, adjacent ganglia and part of the brain were reconstructed after the Born method, and the remainder of the figure was drawn from a dissection of a tadpole of the same age. Enlarged 35 diameters.

the various parts of the labyrinth can be recogniz… dif-
culty, even though they happen to be incomple…
normal relations.

Now if one examines the model… photographs of three of which ar… that the individuality of the … be identified. In model *a*, the … model *b*, the anterior canal is … exists only of a pouch which has no … main cavity; in model *c*, the poster… …ch, while the

anterior and lateral canals are normal. In considering the posture of the canals it is to be noted that the surrounding structures have been left out in Fig. 2, to avoid unnecessary duplication; the three models are all represented in the same relative position as that of the labyrinth in Fig. 1, *i. e.*, the cephalic end is on the right, the caudal end is on the left, the ventral surface is below, and the dorsal surface is above. Thus it will be seen that the lateral canals in all three models are in the same plane; likewise the posterior canals all form the dorso-caudal border of the labyrinth, and the anterior canals form the dorso-cephalic border. The fact that the anterior canal is small in model *b*,[4] and the posterior canal is small in model *c*, gives rise to a false impression of a backward

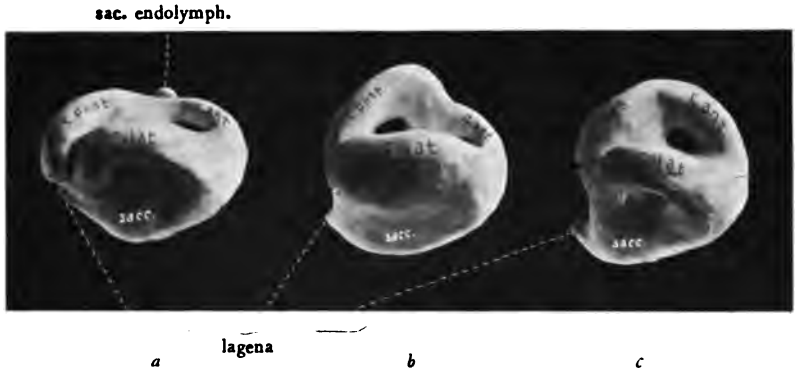

Fig. 2 Reconstructions showing the form and posture developed by three labyrinths one month old, which while primitive ear vesicles were rotated from their normal position so as to lie face inward and upside down. The models are placed so that their planes are parallel with those in Fig. 1. Thus they present a lateral view with the cephalic end toward the right, caudal end toward the left, dorsal surface above, and ventral surface below. Enlarged 50 diameters.

and forward tilting of the vesicle. The saccule and lagena have the same position as in Fig. 1, and the lagena points caudally as it should do. The endolymphatic appendage lies on the median side of the crus commune; in models *b* and *c* it is small, but the tip

[4] This may be due to injury received at the time of operation. Such localized defects are frequently seen. They may involve any part of the labyrinth, and they vary greatly in the extent of the labyrinth wall affected. In one case the entire labyrinth was defective, with the exception of the endolymphatic appendage, which was normal in structure and position, and presented a curious appearance, being attached to the small irregular vesicle representing the labyrinth. Such localization of abnormal development is evidence of the high degree of specialization of the cells forming the primitive ear vesicle.

of it can be seen in model *a*. The acoustic nerve and ganglion are attached to the median and ventral surfaces of the labyrinth, and the nerve connection with the brain appears to be normal.

The conditions found in the three specimens pictured in Fig. 2 are typical of what is found in the other five specimens examined. They vary in the completeness of their differentiation, some of them consisting of only a vesicle with perhaps a single canal pouch, but in all cases the acoustic ganglion is present on the ventro medial surface, and the macular areas can be recognized. The lagena is present in seven out of eight cases. The endolymphatic appendage developed in six out of eight cases. As regards posture, the rule is that the more perfectly the labyrinth is developed the more accurately its posture corresponds to the normal relations. But even in the most imperfect specimens when the endolymphatic appendage appears it is on the medial surface, and the tendency to canal formation is always on the dorso-lateral surface, and the saccule and lagena appear on the ventral surface. This condition of course applies only to vesicles that have been implanted in the acoustic region as was done in all the above cases.

Rotation in One Direction. In four experiments the ear vesicle was rotated 180° around its vertical axis, *i.e*, turned face inward. These specimens were then reared as in the preceding instance, and eventually cut in serial sections. A reconstruction model of one of them is reproduced in Fig. 3, and if it is compared with Fig. 1 it will be seen that although the vesicle was started in its development with invaginated side toward the brain yet the completed labyrinth has the normal posture. A section of the same specimen is reproduced in Fig. 4, showing the labyrinth surrounded by developing cartilage. The acoustic ganglion is connected in normal manner with the brain and sends peripheral fibers to the thickened floor of the saccule. The endolymphatic sac is in its normal position, and the narrow duct can be seen connecting it with the main chamber of the labyrinth directly median to the crus commune. The series through this specimen show that histologically it is practically perfect. Of the other three specimens one was almost equally perfect, another showed some abnormalities in the formation of the canals and the lagena, and the

fourth was quite imperfect, consisting of only a large vesicle with a thickened epithelial floor connected by a few nerve cells and fibers with the brain.

Fig. 3 Reconstruction of a tadpole labyrinth one month old, which when a primitive ear vesicle was rotated from the normal position 180° in one direction, so as to lie with invaginated side toward the brain. A section through the same labyrinth is shown in Fig. 4. Enlarged 55 diameters.

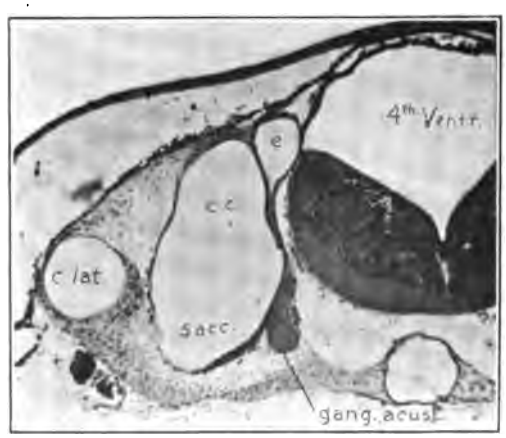

Fig. 4 Section through the membranous labyrinth shown in Fig. 3. It shows that though originally turned face inward it has developed in the normal attitude. *e*, endolymphatic appendage; *c.c.*, crus commune; *sacc.*, saccule; *c. lat.*, lateral semicircular canal; *gang. acust.*, acoustic ganglion. Enlarged 55 diameters.

Transplanted Specimens. The irregularity of form of the six specimens transplanted to the region between the eye and nostril, previously reported,[5] is so great that they give no assistance in solv-

[5] Streeter '06, *l. c.*, p. 557.

ing the question of posture. However, in five cases, which will be presently described, where the ear vesicle was transplanted from the left side to the right side into the place made vacant by the removal of the right ear vesicle, in spite of the fact that these ear vesicles were implanted with haphazard attitude toward the adjacent structures, they nevertheless in each instance developed right-side up, and with the median surface toward the brain, as can be seen in Figs. 5 and 6.

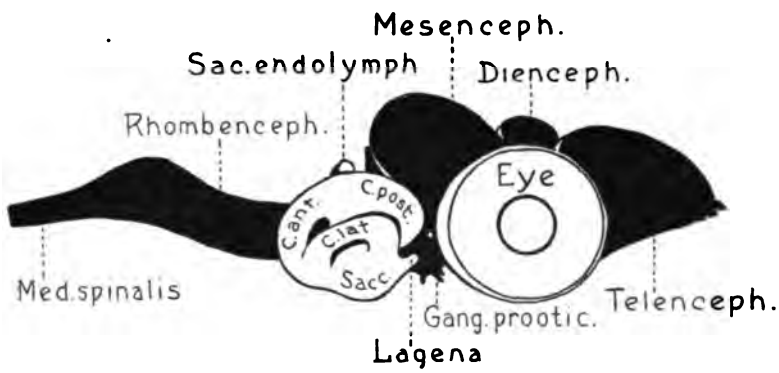

Fig. 5 Reconstruction showing the form and relations developed by a left ear vesicle when transplanted to the right side; it shows that under such circumstances the ear vesicle retains its left-sided characteristics, though it otherwise normally adapts itself to its new situation. A photograph of the same specimen is shown in Fig. 6, *c*.

DETERMINATION OF THE DEXTRAL AND SINISTRAL CHARACTER OF THE EAR VESICLE

The question as to whether the right or left-sidedness of the ear labyrinth is controlled by the environment, or is determined by some intrinsic character of its own constituent cells, is answered in favor of the latter by the fact that if the left primitive ear vesicle, before the time of its complete closure, is transplanted to the opposite side of the embryo it retains its original left-sidedness. In five specimens, at the usual operating stage, the right ear vesicle was removed, and at the same time the left ear vesicle was uncovered and lifted from its natural bed and then placed into the pocket

from which the right vesicle had been taken and allowed to heal. In making the transplantation no effort was made to place the ear vesicles in any particular posture. After keeping the specimens alive for one month they were sectioned and from three of them reconstructions were made of the transplanted ear vesicle together with the adjacent structures. The three labyrinths are shown in Fig. 6, and model *c* is again shown in Fig. 5, with the brain included. It will be seen that in developing they have assumed the normal attitude toward the brain. The endolym-

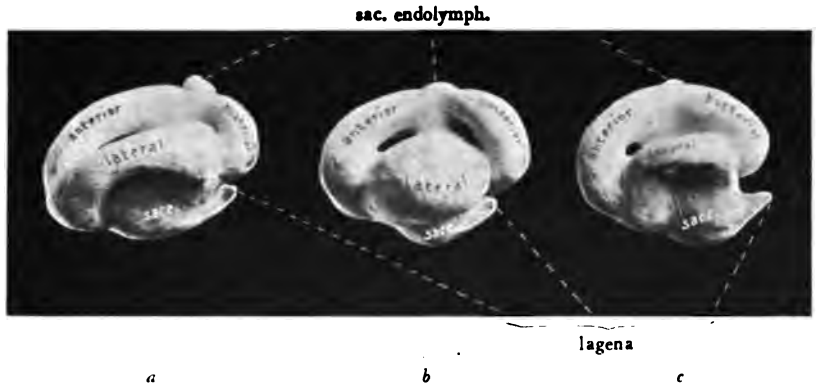

Fig. 6 Reconstructions of three labyrinths which while primitive ear vesicles were transplanted from the left to the right side. They are all represented in the same position as the models in Fig. 2. The model *c* is the same that is shown in Fig. 5. Enlarged 50 diameters.

phatic appendage and the median side of the labyrinth is toward the brain, the semicircular canals are toward the dorso-lateral surface, and the saccule and lagena are toward the ventral surface. But it can at once be recognized that the saccule and lagena point forward toward the eye, and that the anterior and posterior canals are in reversed positions. We thus have a complete mirror image of the right labyrinth, *i. e.*, a left labyrinth. Model *a* possesses three semicircular canals and is almost a normally formed left labyrinth. In model *b* the lateral canal consists of a pouch whose walls did not undergo the customary approximation and central absorption. In model *c* the posterior canal is not pinched off. In each of the models the lagena, saccule, and endolymphatic

appendage are typical, and there is establishment of normal appearing nerve and ganglion connections.

EQUILIBRATION

It was found in the experiments performed a year ago that removal of one or both ear vesicles, just after they are pinched off from the skin, produces in the tadpoles definite disturbances in the development of their power of equilibration. It was found that when a tadpole is deprived of but one ear vesicle he is by virtue of the remaining one able to develop practically normal swimming abilities; but when both ear vesicles are removed the results are more serious, and in that case the tadpole never develops any sense of equilibrium and is never able to swim. The loss is not compensated for by any other organ and the animal lies helpless on the bottom of the dish. With one ear vesicle the tadpole swims practically in normal fashion, and with no ear vesicle he cannot swim at all.

The fact that one ear vesicle is sufficient for the maintenance of equilibrium greatly simplifies the study of this mechanism; it means that one side can be immediately eliminated, and the problem is reduced from a bilateral one to a unilateral one. A series of experiments at once suggested themselves, in which the ear vesicle of one side was to be removed, and then various operative procedures undertaken upon the ear vesicle of the opposite side, and the test of its consequent functional ability was to be the very decisive one of whether the animal could swim properly, or whether it could not swim at all.

In the paper referred to there is described the experiment of transplanting the ear vesicle into a subdermal pocket in front of the eye. When this was done the transplanted ear vesicle continued in its development, and in some instances established a nerve-ganglion connection with the forebrain; but such specimens never gave evidence of functional activity. The failure to functionate was not unexpected, inasmuch as the connections established were at an abnormal situation, and furthermore the vesicles though having developed many essential features of the normal labyrinth

were still quite imperfect in the formation of the separate chambers and the semicircular canals. So this year in carrying out the experiments described in the first part of the present paper the behavior of the specimens was eagerly watched, and the endeavor was made to determine the amount of alteration in position and defectiveness in form that is compatible with functional activity, involving the problem of the correlation between function and morphology. The observations made in the different experiments have been arranged and condensed as follows:

a Left ear vesicle removed; right ear vesicle loosened from skin and rotated, in six specimens around the vertical axis 180° and in eight specimens around both the vertical and transverse axis 180°. As has already been shown these ear vesicles developed into labyrinths of varying degrees of perfection, some being completely normal in form and having apparently normal ganglion and nerve connection with the brain wall. (See Figs. 2, 3 and 4.) The behavior of all the specimens was uniform, both where the ear vesicle was rotated in one plane and where rotated in two planes; at the end of a week after the operation, when with a normally functionating labyrinth they should be able to swim freely and directly, they instead exhibit only irregular movements or spin around in spirals or circles. Their incoördinate movements continue, and at the end of a month there is no improvement; *i.e*, they behave exactly like specimens with both ear vesicles removed. Evidently ear vesicles thus treated do not perform their natural function.

b Left ear vesicle removed; right ear vesicle fragmented by teasing between the points of two needles, the fragments left in place. Ten specimens were treated in this way, and were kept under observation four weeks, during which time they gave no evidence of any sense of equilibrium.

c Right ear vesicle removed; left ear vesicle transplanted to the empty pocket on the right side. Five specimens were operated upon and observed for one month, at the end of which time they were cut in serial sections, and it was found that the ear vesicles had developed into fairly complete labyrinths, but had maintained the characteristics of a left-sided organ. (Figs. 5 and 6.)

Throughout the whole period of observation they had exhibited incoördinate movements, and at the end of that time they were unable to swim. This and the two previous operations indicated that rotation of an ear vesicle, or transplanting it from one side to the other, or fragmenting it was not compatible with the development of its function, in spite of the fact that the ear vesicle proceeded in its development and had become to all appearances almost a perfect labyrinth. In the next experiments less severe treatment was tried.

d Left ear vesicle removed; right ear vesicle uncovered and carefully lifted out and then immediately placed back in its original position, the effort being made to do a minimum amount of injury. Of six specimens all exhibited symptoms of the absence of all sense of equilibrium.

In the experiments *a*, *b*, *c* and *d* there was the possibility of injury to both the nerve-ganglion connection and the ear vesicle. In the following experiments the effort was made to restrict the injury to one or the other.

e Left ear vesicle removed; right ear vesicle uncovered and a fragment cut from the cephalic portion of its wall, care being used not to otherwise disturb the vesicle. Eight such specimens were kept five weeks, and none of them developed any sense of equilibrium, or were able to swim.

f Left ear vesicle removed; right ear vesicle uncovered and a small piece cut from its caudal border, any further disturbance being avoided as in *e*. Eight specimens were operated upon, and after keeping them four weeks none of them could swim properly.

g Left ear vesicle removed; longitudinal incision made through skin on right side just dorsal to ear vesicle, and needle passed down between the neural tube and ear vesicle and moved backward and forward so as to sever its nervous connection without otherwise disturbing the ear vesicle or loosening it from the skin. None of the four specimens studied swimmed properly, though one of them could swim somewhat, but was easily confused by any excitement and then made wild and ill directed movements. It was thought that the ear vesicles in these cases would escape injury; but examination of the specimens when cut in serial sections

showed that they were not perfectly normal. This experiment might be repeated on a larger number of specimens and still greater care used in severing the nerve connection, in which case a perfect labyrinth could doubtless be obtained.

h (Rana catesbiana) Left ear vesicle transplanted into another specimen, in a subdermal pocket in the region of the prootic ganglion between the right eye and ear vesicle, thus the host had three ear vesicles, two being on the right side. Twelve days after the operation three out of four specimens so treated exhibited incoördinate movements. Here we have to consider the crowding out of position of the normal right ear vesicle by the one transplanted near it.

i Left ear vesicle removed; fine needle passed through the skin so as to make a small puncture in the right ear vesicle; on withdrawal of the needle the edges of the wound immediately close and there is no loss of cells from underneath or from the skin itself. Of four specimens at the end of one month three were able to swim, and this demonstrated the functional ability of an ear vesicle thus treated.

j Left ear vesicle removed; small section of the covering skin removed so as to expose the right ear vesicle, but otherwise it is not disturbed and the nerve ganglion connection is left intact. Five specimens were kept under observation for one month, and four of them behaved throughout like those possessing one untouched normal ear vesicle; except for slight incoördination brought out by excitement they could swim properly.

On bringing together the results of these experiments, it becomes immediately apparent that almost any operative procedure carried out on young larvæ in the region of the ear vesicle seriously interferes with the development of the function of that organ. It is possible to lift a skin flap and expose it, and to make a needle puncture in it without destroying its subsequent usefulness; but any operation involving a loss of part of its wall or disturbing its position and nerve-connection with the brain causes apparently complete loss of function. The functional disturbance is out of all proportion to the histological condition. There may be a labyrinth that to all appearances is perfectly formed and that seems to

have a normal nerve ganglion connection with the brain at the proper place, and yet the specimen may not have given signs of any functional activity on the part of that organ.

Spemann[6] is doubtless mistaken in attributing the disturbance in equilibrium simply to the alteration in the planes of the canals. He reports some experiments in which at an early stage a skin flap was turned back, and the ear vesicle taken out and replaced in various positions; and in such specimens he observed faulty equilibrium, and on sectioning his material the vesicle seemed to lie in an abnormal position, and this he assumes to be the cause of the abnormal movements observed. On the one hand, wax plate reconstructions of misplaced ear vesicles show that in my cases they regain their proper position, and the canals eventually lie in their normal planes; the specimens nevertheless continue to make incoördinate movements. On the other hand, in those experiments where the normal position of the vesicle, as regards the planes of space, was undisturbed the results were equally serious. My own experiments suggest that the difficulty lies not so much with the end organ as with the central connections, and perhaps further experiments in that direction would furnish additional information upon this subject.

CONCLUSIONS

The primitive ear vesicle of the tadpole may be loosened from its normal position and rotated in various directions, so that its axes lie in abnormal planes, and notwithstanding such interference it eventually develops into a labyrinth which is right side up and exhibits the normal relations to the brain and the surrounding structures. When transplanted to the opposite side of the body, if placed in the acoustic region, it likewise assumes a normal posture. Judging from these facts, the posture of the labyrinth is controlled by its environment.

The "laterality" of the labyrinth is determined before the closure of the ear vesicle. When the left ear vesicle is transplanted

[6] Spemann, H., '06: Ueber embryonale Transplantation. Verhandl. der Gesell. Deutscher Naturf. u. Aerzte. 78 Vers. Stuttgart.

to the right side it retains its characteristics as a left-sided organ, though it otherwise adapts itself to its new position in a normal manner.

The functional disturbance, in experiments on the ear vesicle, is out of all proportion to the histological appearances; any operation carried out in the acoustic region involving a loss of part of the wall of the ear vesicle, or disturbing its position, or nerve connection with the brain results in faulty equilibrium; absence of function was observed in cases where the labyrinth and its nerve connections seemed to have attained perfect histological development.

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The Lymphatic Drainage of the Faucial Tonsils.

BY

GEORGE BACON WOOD, M.D.,

OF PHILADELPHIA.

FROM THE

AMERICAN JOURNAL OF THE MEDICAL SCIENCES,

AUGUST, 1905.

Extracted from the American Journal of the Medical Sciences, August, 1905

THE LYMPHATIC DRAINAGE OF THE FAUCIAL TONSILS.[1]

By George Bacon Wood, M.D.,
of Philadelphia.

(From the Wistar Institute of Anatomy, University of Pennsylvania.)

That various micro-organisms may gain access to the general system through the tonsillar tissues of the throat has been so firmly established that it is not necessary again to demonstrate the absorbing properties of the tonsils and to point out the various infections which may enter through this portal. The fate of the micro-organisms which have entered the parenchyma of the tonsil is dependent upon two factors: first, the pathogenic potency of the germ itself, and, second, the vital resistance offered by the tissues to its invasion. If a non-pathogenic germ enters a crypt it is destroyed probably by the phagocytosis of outwandering polymorphonuclear leukocytes. On the other hand, if the vitality of the germ is sufficiently great, it lives and may penetrate through the epithelium into the interfollicular tissue.

It has been proven by several very thorough and capable investigators that foreign bodies in the crypts can pass through the epithelium into the interfollicular tissue. This absorption is probably dependent upon two factors: first, the action of the muscles of the throat during their various physiological movements, and, second, the presence of a lymph current in the tonsil. The palatoglossal muscle and the palatopharyngeal muscle during deglutition compress the faucial tonsil and force the centrally lying movable bodies toward the periphery. If the foreign body is at the bottom of the crypt it is pushed in an outward direction, and, finding no appreciable barrier in the differentiated epithelium, passes into the tonsil parenchyma. The second factor in the absorption of foreign bodies from the crypts is the constant production of lymphoid cells in the follicles. The interfollicular tissues of the tonsils consists of a fine connective-tissue reticulum interspersed with numerous lymphoid cells. These lymphoid cells may be looked upon as being in constant motion, passing from the follicles in the direction of least resistance. If the cellular metamorphosis of the epithelium has weakened this barrier so that the crypt is in the direction of least resistance, the lymphoid cells break through and pass into the crypt, but more commonly the lymphoid cells pass into the lymph spaces

[1] Candidate's thesis. American Laryngological Association, June, 1905.

which terminate in the trabeculæ of the tonsil and which in their turn empty into the efferent lymphatics of the tonsil. Therefore, the production of lymphoid cells in the follicles causes what we might term a lymphoid current, and this lymphoid current, except when there is an accidental rupture of the cryptal epithelium, is toward the connective-tissue trabeculæ of the tonsil and thence through the lymph radicles to the periphery. A foreign body having gained access to the interfollicular tissue must be influenced by this current. I understand thoroughly that in advancing this idea, I am open to criticism for theorizing about a scientific subject. It seems to me, however, that the above is a probable explanation of the mechanism of tonsillar absorption and helps to substantiate the theory that the faucial tonsil is more important as a source of infection than is the lymphoid tissue on the lateral folds and posterior pharyngeal wall of the pharynx. As regards the absorption of living virulent micro-organisms, it must be remembered that bacteria are not inert foreign bodies, but possess certain vital properties which influence their entrance into living tissue. When uninfluenced by extraneous forces a micro-organism invades living tissue by two methods: first, if it belongs to the motile class the individual bacterium by its own locomotion may carry itself to some more or less distant spot, and, second, micro-organisms may invade tissue by the very act of their growth. This last is the most important method by which the pathogenic germs gain entrance into their host.

It is practically impossible to establish with absolute certainty the relative importance of the different lymphatic masses in the throat as gates of infection. Circumstantial evidence, however, both clinical and experimental, tends to show that the larger the lymphatic mass and the deeper its crypts, the more readily do pathogenic germs pass through the tonsillar tissues to the tonsillar efferents.

The research outlined in this paper is a preliminary report of a series of investigations concerning the lymphatic anatomy and drainage of the tonsils and surrounding portions of the throat. My first investigations concern the faucial tonsil, because in the etiology of infections it is the most important tonsillar structure, and I have not been able to find any reliable published data concerning the glands that receive its efferent lymphatics.

The lymphatic glands of the neck constitute one of the most important chains of glands in the whole body and is probably the chain which is most frequently diseased. The infection which inoculates a lymph gland may possibly come through its efferent lymphatics by a retrograde thrombosis, but much more frequently it follows the current of the lymph stream and enters through the afferent vessels. In the large majority of cases the infection of the cervical lymphatics comes from the nose, mouth, or throat. As a general rule, the efferent lymphatic vessels from a given structure

drain into that lymphatic gland which is nearest it, and, therefore, to understand the importance of the different structures of the nose, throat, and mouth as portals of infection it is essential to have clear in our minds the topographical relations of the deep and superficial lymph glands.

The cervical glands of the neck are divided into two main groups, the superficial or collecting glands and the deep or terminal glands. The superficial are arranged as a sort of collar around the upper part of the neck, with a few irregular extensions. This pericervical glandular circle is composed of the following subgroups:

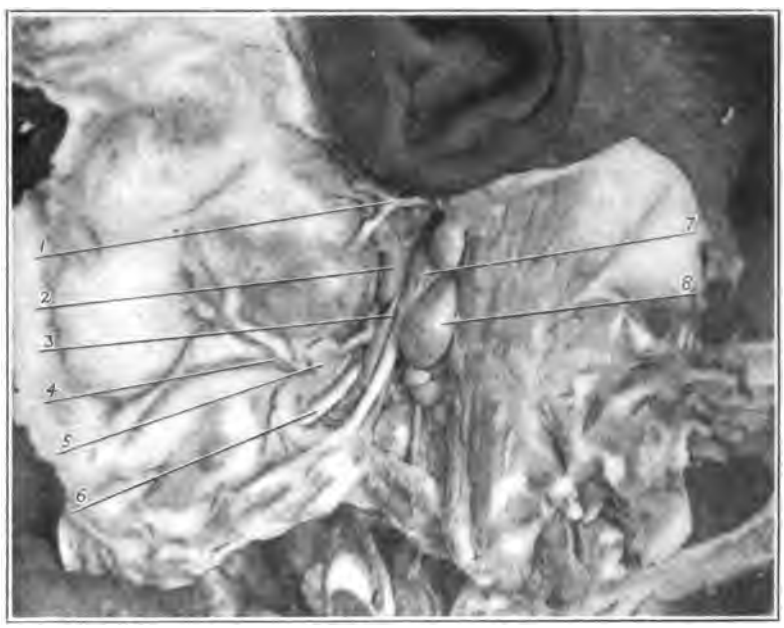
1. Suboccipital group and aberrant glands of the nape of the neck.
2. The mastoid group.
3. The parotid and subparotid groups.
4. The submaxillary group with the facial glands as an offshoot.
5. The submental glands.
6. The retropharangeal glands.

The terminal or deep glands of the neck form a perpendicular chain beneath the sternocleidomastoid muscle. This main set of glands is flanked by several secondary chains of less importance. The superficial glands constituting the pericervical collar receive their afferents from those portions of the external and internal head, which are in more or less close relation with the individual group. The efferents of all these collecting glands empty into the deep chain. The deep cervical lymphatics or the substernocleidomastoid glands constitute in appearance a continuous chain, but for convenience sake they may be divided into an external and internal group. The external glands are placed posteriorly and rest indiscriminately on the insertions of the splenius, levator anguli scapulæ, and scalene muscles, and they are usually continuous with the glands occupying the supraclavicular triangle. The internal glands of the substernocleidomastoid group may be termed the internal jugular chain, because they rest either directly on the internal jugular vein or are immediately adjacent to its external border. The glands of this subgroup are larger than the glands of the external, and some of them are fairly constant in their position. One or two large glands are constantly found beneath the posterior belly of the digastric, just above the spot where the thyrolingual facial vein opens into the internal jugular. A few glands are sometimes found between the internal jugular and the prevertebral muscles. These two main groups of deep glands, the external and internal chains, are united by a great many anastomoses. The external glands receive their afferents from the posterior part of the head and neck, while those of the internal group receive their afferents from the anterior portion of the neck and head, from the mouth, nose, pharynx, larynx, and thyroid body. While the majority of the afferents of the deep glands of the neck are the efferents from the superficial glands, quite

a number of lymph vessels pass directly from the lymph radicles of the throat and nose to the deeper chain of glands. For instance, the majority of the lymph vessels from the vault of the pharynx drain into the retropharyngeal glands, but some of them pass directly to the upper glands of the deep cervical chain without any interruption.

The method of injection employed in the present research was essentially that devised by Gerota. It consisted in rubbing up Berlin blue with pure spirits of turpentine until it assumed the consistency of a rather thin, syrupy liquid. A small quantity of ether is added

FIG. 1



Dissection of the neck of a child, showing an enlarged tonsillar lymph gland and its relation to the digastric and stylohyoid muscles: 1, facial nerve; 2, external carotid artery; 3, stylohyoid muscle; 4, facial artery; 5, submaxillary lymph gland; 6, hypoglossal nerve; 7, posterior belly of the digastric muscle; 8, tonsillar lymph gland.

and the mixture filtered through chamois skin. The coarser granules of the Berlin blue are held back in the filter while a clear, thin, blue liquid passes through as the filtrate. Gentle agitation of the fluid while it is filtering ensures a better mixing of the turpentine, ether, and Berlin blue, consequently a deeper color to the filtrate. The injecting apparatus consisted of a fine glass needle drawn out of a glass tube and attached by a piece of rubber tubing to the nozzle of an easy-working syringe holding about 10 c.c.

The glass needle was inserted beneath the mucous membrane covering the tonsil and held very carefully in its place, while an

assistant made gentle but gradually increasing pressure, according to the ease with which the fluid entered the tonsil. The amount injected was regulated somewhat by the amount of resistance felt in the syringe and also by the distention of the tonsillar tissues. If the injection was properly made the tonsil gradually swelled out becoming two or three times its normal size and of a deep-blue color. The blue fluid in such injections must not be forced in hurriedly, as a certain amount of time is necessary for it to find its way into the lymph spaces and thence through the lymph radicles to the

FIG. 2

Dissection of the neck of an adult, showing enlarged internal jugular lymph glands: 1, parotid gland; 2, masseter muscle; 3, facial nerve; 4, facial artery; 5, submaxillary lymph gland; 6, submaxillary salivary gland; 7, thyrofacial lingual vein; 8, internal jugular vein; 9, brachial plexus of nerves; 10, superficial cervical plexus; 11, spinal accessory nerve; 12, internal jugular lymph glands.

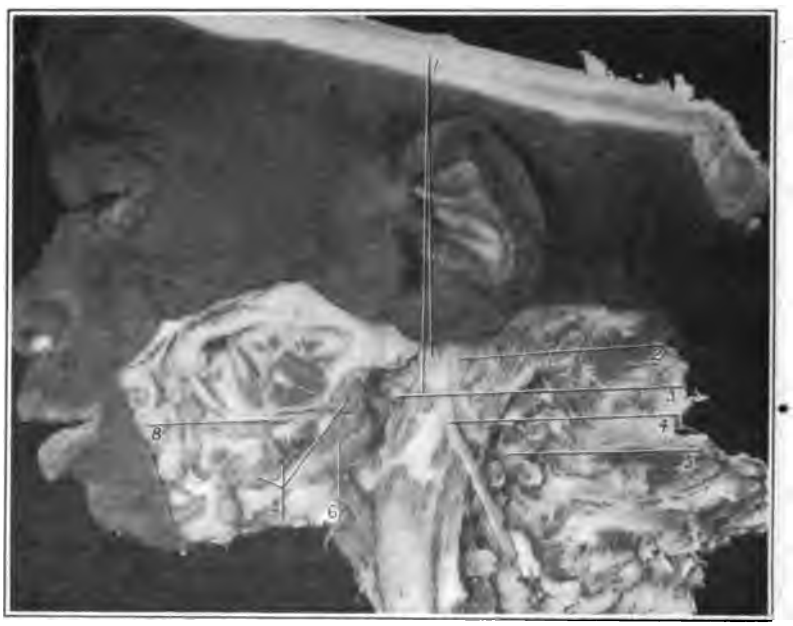
lymph vessels. In injecting the tonsil, it frequently happens that the fluid returns through the crypts. In the case of this regurgitation being so free that internal pressure cannot be exerted, the point of the needle is to be changed until a position is found from which the injected material does not so readily gain access to the crypts. After the injection has been made the excess of material should be washed from the surface by a stream of cold water. Gentle massage of the tonsil with the finger tends to force the injected fluid farther into the tissue. It is best to leave the fresh material in plain

water for several hours before fixing it in 10 per cent. formalin. After fixing the specimen the dissections may be made and the injected gland sought for, special care being exercised to avoid wounding any of the injected lymph vessels

Eight injections were made, some of them resulting in failure, but the majority showing distinctly that the tonsillar lymph follows a fairly constant route. The direction of this drainage as established by these injections is as follows:

The lymph vessels pass from the external portion of the tonsil through the peritonsillar connective tissue, the pharyngeal aponeu-

FIG. 8



Superficial dissection of Specimen No. VI., showing enlarged tonsillar lymph gland projecting just beyond the edge of the sternocleidomastoid muscle: 1, external jugular lymph glands; 2, great auricular nerve; 3, tonsillar lymph gland; 4, external jugular vein; 5, external glands of the substernomastoid group; 6, submaxillary salivary gland; 7, submaxillary lymph glands; 8, facial artery.

rosis, and the superior constrictor of the pharynx, and, as one or two or more fine small vessels run obliquely in a downward, posterior, and outward course, passing below the facial artery. Bending more posteriorly the lymph vessels next run between the internal jugular vein and the stylohyoid muscle, reaching finally the superior surface of an enlarged lymph gland, placed just beneath the anterior border of the sternocleidomastoid muscle, where it is crossed by the posterior belly of the digastric muscle. The efferent vessels from this gland are generally two or three in number, and pass into the neighboring glands of the internal jugular group. Further

anastomoses which connect the lower glands of the internal jugular group with those receiving the tonsillar drainage form a complete lymph channel, through which the tonsillar lymph finally empties into the jugular lymph trunk.

In none of the preparations did the injected fluid enter into superficial glands, except in one case, where there was an aberrant gland lying on the facial artery near its origin. This gland seemed to be simply an interrupting nodule in the course of the tonsillar efferents. The statement in some text-books on anatomy that the tonsil drains

FIG. 4

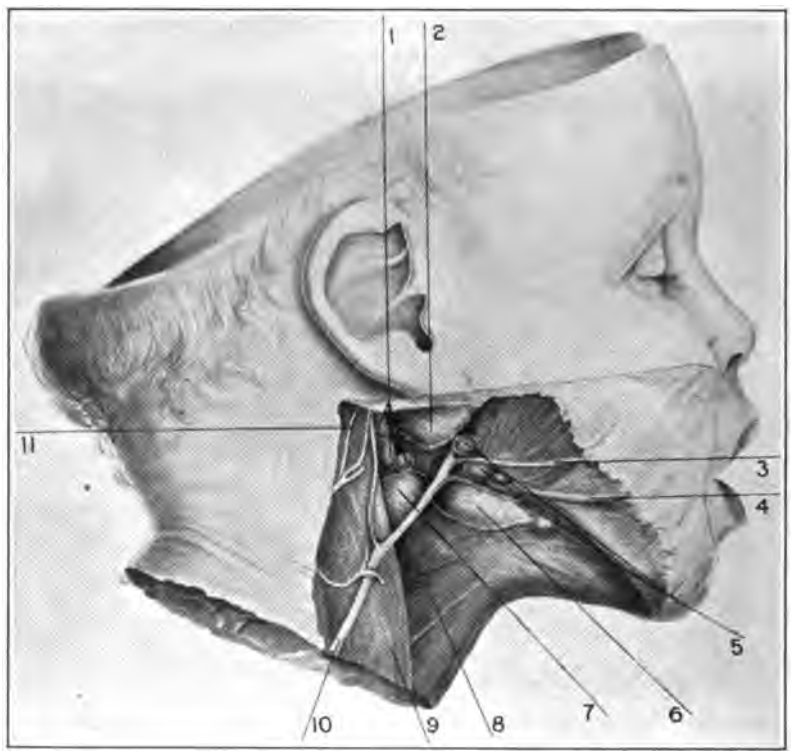
Deep dissection of Specimen No. VI., showing the tonsillar lymph gland and its relation to the substernomastoid group: 1, reflected sternocleidomastoid muscle; 2, tonsillar lymph gland; 3, spinal accessory nerve; 4, branch of cervical plexus; 5, internal jugular vein; 6, omohyoid muscle.

into the posterior gland of the submaxillary group would seem to be absolutely erroneous and very misleading.

Clinically, the gland which becomes enlarged during tonsillar infection appears to be superficial and possibly it is this appearance that has led to the belief that the posterior gland of the submaxillary group is infected through the tonsils. The tonsillar gland, if I may be pardoned for using the term, is placed external and slightly anterior to the internal jugular vein and is embedded in loose areolar tissue containing more or less fat. Consequently, enlargement of this gland means its dislocation outward and forward, and especially

so if the other glands of the internal jugular group become subsequently enlarged; also the action of the substernocleidomastoid muscle would tend to force it anteriorly. If an enlarged gland found at the angle of the jaw belongs to the deep substernocleidomastoid group, it may be pushed back under the muscle of the same name. In cases of enlargement of the submaxillary glands such as result from infection from carious teeth, the glands are found along

FIG. 5

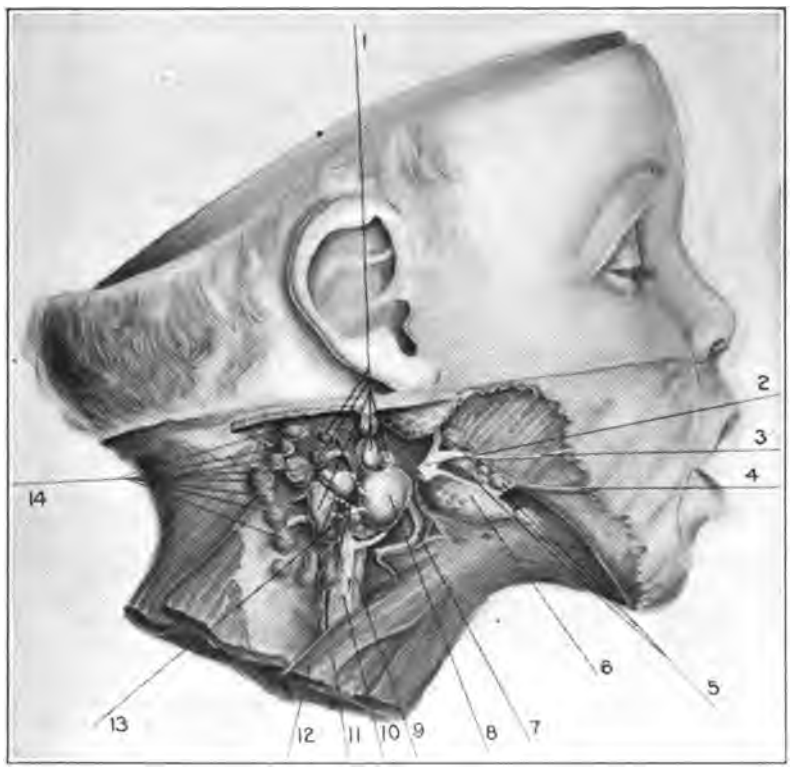


Superficial dissection of Specimen No. II., showing portion of tonsillar lymph gland and its relation to the sternocleidomastoid muscle and the external jugular vein: 1, lymph glands of the internal jugular group; 2, parotid gland; 3, facial vein; 4, facial artery; 5, submaxillary lymph glands; 6, submaxillary salivary gland; 7, tonsillar lymph gland; 8, omohyoid muscle; 9, sternocleidomastoid muscle; 10, external jugular vein; 11, great auricular nerve.

the edge of the jaw and cannot be displaced backward and only slightly downward. In the description of the anatomy of the cervical lymphatics previously given it was stated that where the posterior belly of the digastric muscle crossed the sternocleidomastoid there were constantly found one or two large glands. In a child one week old in which I was able to trace the course of the tonsillar efferent vessels this gland was very little larger than many more of the external jugular group. In children who have reached six or more months

of age this gland was generally twice or three or four times larger than any other, and in every injection of the tonsil it was this enlarged gland that primarily received the blue injecting fluid. Does it not seem possible that this enlargement consequent to birth may be due to the absorption of toxins through the faucial tonsils?

FIG. 6



Deep dissection of Specimen No. II., showing position of tonsillar lymph gland and its connection with other glands of the substernomastoid group: 1, internal substernomastoid lymph glands; 2, external jugular vein; 3, facial vein; 4, facial artery; 5, submaxillary lymph glands; 6, submaxillary salivary gland; 7, superior thyroid artery; 8, tonsillar lymph gland, 9, common carotid artery; 10, internal jugular vein; 11, omohyoid muscle; 12, jugular lymph trunk; 13, efferent vessels of tonsillar lymph gland; 14, external substernomastoid lymph glands.

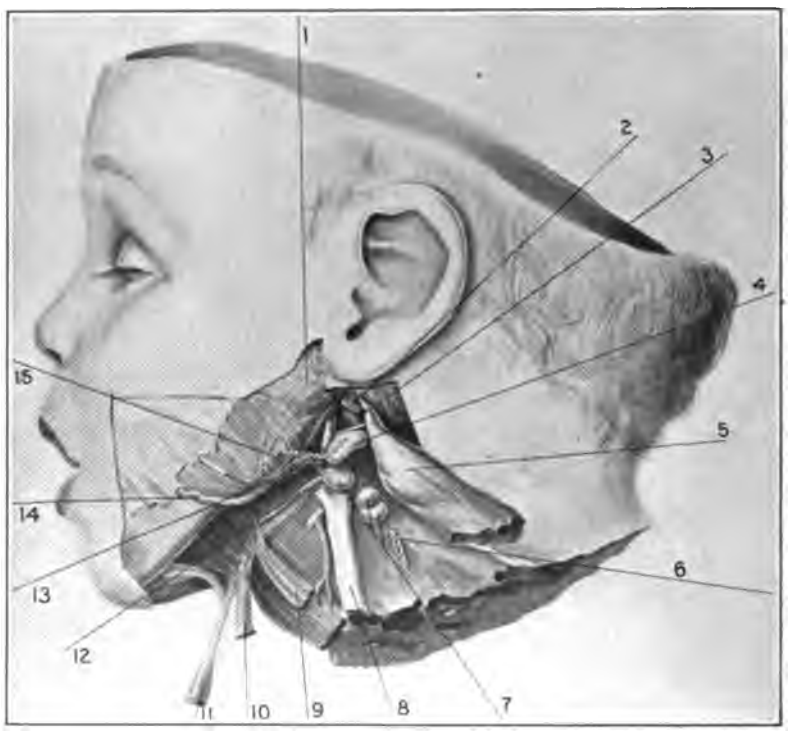
I have chosen to call the lymphatic gland which receives the efferents of the tonsil the *tonsillar lymph gland.* This term is not sufficiently comprehensive, because most probably other lymph vessels from the throat, besides those from the tonsil, drain into this gland, but the most important infections of the cervical lymphatics originate through the tonsil, and because of the importance of the tonsil in the origin of infections, I feel that the term tonsillar is a most

appropriate one. Certainly, it is convenient to be able to easily designate so important and constant a lymphatic gland.

Following are the descriptions of the individual injections of the tonsils. The technique was practically the same in all of them.

SPECIMEN I.—Child about six months old. The injection in this case failed probably because there was some obstruction in the injecting needle. Dissection of the neck showed hypertrophy of the tonsillar lymph glands.

FIG. 7



Deep dissection of Specimen No. IV., showing the efferent lymph vessels of the faucial tonsil entering the tonsillar lymph gland. The posterior belly of the digastric muscle and the whole of the stylohyoid muscle have been reflected downward: 1, parotid gland; 2, stump of the stylohyoid muscle; 3, stump of the posterior belly of the digastric muscle; 4, tonsillar lymph gland; 5, reflected sternocleidomastoid muscle; 6, brachial plexus of nerves; 7, substernomastoid lymph glands; 8, internal jugular vein; 9, omohyoid muscle; 10, stylohyoid muscle; 11, posterior belly of the digastric muscle; 12, hypoglossal nerve; 13, efferent lymph vessel of the faucial muscle; 14, facial artery; 15, position of faucial tonsil.

SPECIMEN II.—Child about six months old. The injection in this case was very successful. The tonsil itself became enlarged and there was a slight overflow of the fluid toward the faucial pillars, the blue color, however, was limited to the fauces. A few injected lymph vessels were seen running posteriorly into the sinus pyriformis and anteriorly toward the base of the tongue. The dissection of the

neck showed that the blue fluid had entered a slightly enlarged lymph gland situated just below the point where the anterior border of the sternocleidomastoid muscle is crossed by the posterior belly of the digastric. The gland was superficial to the digastric muscle just above the facial vein, where it joins the external jugular. A deeper dissection demonstrated that the injected gland belonged to the anterior group of the upper deep cervical lymph glands and had three efferent vessels, which also contained the blue injecting fluid. The connecting lymph vessels between this gland and the jugular trunk were dissected out. The most direct route showed that the lymph current from the tonsil, in order to enter the jugular trunk, had to pass through two or more glands after leaving the tonsillar lymph glands. In this specimen, however, I was unable to find the efferents running from the tonsil to the tonsillar lymph gland. Figs. 5 and 6 were made from this specimen.

SPECIMEN III.—Child one week old. Only a small quantity of fluid was injected into the tonsil. The dissection showed a slight injection of the glands situated just below the posterior belly of the digastric.

SPECIMEN IV.—Child one week old. A rather full injection of the tonsils was made and there was some infiltration of the surrounding mucosa. This injection was very instructive, as by careful dissection lymphatic vessels could be traced running from the infiltrated tonsil directly to the gland situated just below the posterior belly of the digastric beneath the anterior border of the sternocleidomastoid muscle. This lymph gland was considerably injected, but the importance of the specimen was in the demonstration of the efferent lymphatics of the tonsil. The course which the lymphatic vessels took was as follows: Leaving the tonsillar mass and running posteriorly and inferiorly, they pass slightly outward beneath the facial artery, running along the upper border of the lingual vein, then turning directly posterior, they pass between the stylohyoid muscle and the internal jugular vein, reaching the upper surface of the tonsillar lymph gland. Fig. 7 was made from this specimen.

SPECIMEN V.—Child about three months old. Too much fluid was injected in this specimen, and, although the tonsillar lymphatic gland and its efferents contained the blue solution, the extravasation through the connective tissue of the neck was so great as to make it impossible to recognize the topography.

SPECIMEN VI.—Child about three months old. This injection was a most successful one. Quite a large amount of fluid was injected in this case without any extravasation. In the throat the fluid was limited to the confines of the tonsil and the dissection showed that it had followed the lymph vessels through six or more of the upper deep cervical glands. The efferents from the tonsil could be traced passing in a direction very similar to that described under Specimen IV. There were two or three lymph vessels which ran closely together in

a more or less parallel course. At first they passed obliquely downward, posteriorly, and outward until they reached the facial artery. Running beneath this and internal to the stylohyoid muscle and the posterior belly of the digastric muscle, they entered the internal side of the tonsillar lymph gland.

In closing, I desire to express my thanks to Dr. James M. Stotsenburg and especially to Dr. Harold D. Senior for their skilful aid in making the injections.

CONCERNING THE WISTAR INSTITUTE OF ANATOMY

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June 1, 1907

REPORTS.

CONCERNING THE WISTAR INSTITUTE OF ANATOMY.

On Monday, March 4, the Board of Managers of the Wistar Institute of Anatomy held its annual meeting to consider the work of the year just closed and to approve plans for the year 1907.

As the principal aim of the Institute is to serve the science of anatomy it may be of interest to present here a brief excerpt from the Director's report, which reviewed the essential points of the year's activities, gave a full statement of the Institute's finances and suggested plans for the future.

The general plan of the Institute, outlined early in 1905, was to organize an Advisory Board of Anatomists, which should be representative of the active research anatomists of the country, and by the assistance of such a Board to determine from time to time the details of a plan which would enable the Wistar Institute, with is modest but steadily increasing endowment, to render the greatest aid to the science of anatomy. The plan further contemplated the organization of a local scientific staff, the assembling of such laboratory equipment as would be required, not only in the ordinary research work of the Institute, but also from time to time in extraordinary researches originating here or elsewhere, and the constant addition to the museum of materials of actual scientific value.

The plan met with the approval of those anatomists who were consulted.

The Advisory Board at its first meeting prepared definite suggestions for the establishment of a local research staff and for placing the Institute in the proper relation to research anatomy.

The following committees were appointed:

On Neurology and the Establishment of Relations with the International Association of Academies: Dr H. H. Donaldson, Chairman; Dr. L. F. Barker, Dr. F. P. Mall, Dr. J. P. McMurrich, Dr. C. S. Minot.

On the relations of the Wistar Institute to American Anatomists: Prof. S. H. Gage, Chairman; Dr. G. Carl Huber, Dr. G. A. Piersol.

On Comparative Anatomy and Embryology: Dr. G. S. Huntington, Chairman; Dr. E. G. Conklin, Dr. F. P. Mall.

Aside from the routine duties in connection with a public museum almost the entire year 1905 was spent in developing the plans suggested.

At the beginning of 1906, the records of which we have just officially closed, the Institute secured as its research chief in neurology Dr. Henry H. Donaldson and the real work was begun. Later in the year the scientific staff was augumented by the election of Dr. George L. Streeter and Dr. S. Hatai as Associates in Neurology, making a total of seven on the staff.

The internal readjustment of the affairs of the Institute and the equipment of the laboratories have progressed steadily during the year.

In April, 1906, the second meeting of the Advisory Board was called, and the results of their discussion are briefly set forth in the following suggestions:

1. That the Institute initiate a study of racial anatomy of the brain and cooperate with foreign institutes to secures brains of other races.

2. That the Neurological Committee be requested to consider means for the further organization of neurological workers in this country.

3. That it be recommended to develop a staff of expert laboratory assistants, such as draughtsmen, modelers. and technicians, to facilitate the mechanical work of research.

4. That investigators be admitted from time to time by the Director to the full advantages of the laboratories as guests for such periods as may be determined upon.

5. That whenever opportunity offers of obtaining specially desirable material for the study of comparative anatomy and embryology this should be secured and preserved for future use.

Of suggestion No. 1 our report will show an addition to our collection of 77 human brains, representing the racial anatomy of this organ. Nine of these specimens are worthy of special mention, but for obvious reasons their identity must be withheld. This is an important part of our work, and the large number collected during the year is due primarily to our relations with other institutions. The collection of human brains representing race types is perhaps one of our most important and immediate duties as a museum, for this will be impossible in the not very distant future.

Concerning recommendation No. 2, it may be said that the Neurological Committee has made progress. Its work is not yet completed and cannot be reported in full. An effort is being made to connect our Institute with other institutions which will furnish opportunities for clinical work and the collection of another class of material.

Recommendation No. 3 is perhaps one of the most important for the success of our laboratory and can be carried out when our income is augmented or when we are able to economize in some other direction.

To make our facilities complete and satisfactory we must have laboratory assistants, draughtsmen, modelers, and technicians as recommended. It is just here that most laboratories are weak and are unable to furnish the investigator with that perfection of apparatus and technical assistance which will enable him to complete his researches. This part of our force will be developed as rapidly as possible. It must be said, however, that such equipment should come slowly as the work demands it, otherwise there will be a useless waste of energy and funds.

Investigators are admitted to our laboratories as proposed in recommendation No. 4. A number have availed themselves of the opportunity during the year.

Concerning recommendation No. 5 it may be said that we have collected and stored material for comparative anatomy whenever the opportunity presented itself. The museum is especially rich in certain lines and some of this material has been sent to investigators in other laboratories.

As a result of our effort to create here a Central Anatomical Institute and of our decision to follow neurology for the present as our major subject we received, in February, from the Central Commission for Brain Investigation, through the Imperial Academy of Sciences at Vienna, a formal recognition of the Wistar Institute as an international central institute for brain research in America. Hereafter all work in America in cooperation with the Central Brain Commission may be communicated through the Wistar Institute.

In May a meeting of this Commission was held in Vienna. Professor Donaldson, as a member and the Director of the Institute, by special invitation, attended this meeting. The actions of this Commission, soon to be published by Professor Waldeyer, will be of interest to neurologists.

Naturally our museum growth has been greatest in neurological material, and while not great in numbers every specimen is significant. In the museum catalogue during the year 148 entries have been made, comprising 27 series of neurological preparations containing 2568 slides; 23 series of shad embryos, 6 reconstructions of the developing shad's heart, 2 models of embryo shad (the series of embryos, reconstructions and models all belonging to one research), and 77 human brains of special value and interest, the remaining entries consist of a variety of anatomical material. Of the human brains received 63 were negro brains presented

by Professor Franklin P. Mall, of Johns Hopkins University. They represent a series which has been carefully studied for certain race characteristics (American Journal of Anatomy, Vol. V, No. 4,) and are now held for future investigations on the brain of this race. Nine brains of special individual interest have been received during the year. In addition to these the museum has acquired a number of special preparations presenting normal human anatomy, which add to the attractiveness of this part of the museum, though they are not of special research significance.

The equipment of the laboratories has required no small amount of attention. Such apparatus as may be purchased in the markets has been supplied. The best forms of Zeiss microscopes, photographic lenses, the newest types of microtomes, and the many other appliances which go to make up a laboratory equipment have been furnished.

Among the special devices which have been built in our own shop may be mentioned the projection and photomicrographic apparatus. This instrument is designed to meet the requirements of the anatomical laboratory where drawing or photographs from sections or other objects may be required and obtained with the least possible effort and minimum amount of time, or where the object may be studied directly and measurements made by means of this apparatus without the photographic processes. The apparatus is always in working order, no rearrangement of cumbersome pieces being necessary to operate it. The apparatus is mounted in a dark room, with a developing room adjoining, directly in one of our main laboratories so that the work of preserving, preparing, and photographing or drawing a specimen may be done on the same floor within a radius of a few feet.

Although we now have two large microtomes the reconstruction of a new brain-cutting microtome for much finer and better work is underway. This will add to our facilities for producing valuable series of brain sections.

As anatomy has been studied by the various mechanical means of analyses there now remains the chemical means of attack. For this purpose the Institute has recently equipped a bio-chemic laboratory supplied with all the necessary apparatus, much of which was constructed in our own shop. I mention in some detail these bits of special equipment to emphasize the fact that our shop facilities make it possible to supply any apparatus which cannot be purchased in the market but which may be demanded for special research work.

Concerning library facilities it is not necessary for me to say that

Philadelphia is unequaled in this respect, the magnificent library of the Academy of Natural Sciences, of the American Philosophical Society, of the College of Physicians, and of the University of Pennsylvania, not to mention a number of other large libraries are all accessible to the members of our laboratory. Of the Institute's library it may be said that here are to be found all the principal journals and reference books required in anatomical work. This year 46 new volumes were added to our library, making a total to date of 1486 bound volumes. We have received 41 periodicals and 14 books issued in parts, 55 in all. The reprints have all been carefully catalogued under both author and subject.

One of our most important accessions is, perhaps, a complete set of the bibliography cards relating to microscopy, physiology, and anatomy, issued by the Concilium Bibliographicum. They are divided as follows: Microscopy, 3230 cards; physiology, 16,098 cards, and anatomy, 28,056 cards, making a total of 47,384 cards correctly filed and accessible. These cards represent bibliographical data in the three subjects named from 1898 to date, excepting in physiology, in which branch the publication was discontinued from 1899 to 1904, but was resumed in 1905. This set of cards of the Concilium Bibliographicum is, I believe ,the only set in Philadelphia, and is, of course, open to anyone who may desire to use it.

While every book has been accessioned, I regret to say that on account of lack of time we have been unable to complete our card catalogue of the library. This will be taken up during the summer months when there are less demands from other directions upon the time of the librarian.

The neurological library belonging to Professor H. H. Donaldson has been placed in the Institute for the use of investigators in the laboratories. The library consists of more than 1000 bound volumes and 4000 reprints and subscribes to 14 scientific journals. It forms a most valuable acquisition to our working equipment.

In this connection I must also mention the very valuable library, consisting of some 4000 volumes, largely scientific, willed to the Institute by General Wistar and which has been placed in dust-proof cases in a specially prepared room at the Institute.

It is with pardonable pride that I record the results of our efforts to establish research in our laboratories and make our museum subservient thereto. Investigations for the present are directed to neurology, and the chief resources of the Institute are being expended to develop research in this department; there is no desire, however, or effort made to limit researches to this field, should any investigator desire to pursue in our

laboratories investigations in any other field. In neurology, under the direction of Professor Donaldson, some fourteen pieces of research are underway in our own laboratory while a number of others are being prosecuted elsewhere, also under Professor Donaldson's direction.

In pursuing researches in neurology it is essential to have an abundant supply of fresh material, and a single type of animal tends to increase the accuracy of deductions. It is for this reason that we have established a colony of Albino rats which are bred to a standard of weight and size and furnish material of the proper kind. This colony comprises several hundred animals. In addition to this we have established also a colony of opossums (*Didelphys virginiana*) the only representative of its family in America and presenting an extremely interesting anatomy from the neurological and embryological standpoint. These two forms will furnish abundant material of its kind for laboratory use.

Every effort will be made to strengthen our relations with other laboratories and to assist in every possible way in promoting researches in anatomy. To this end we have attempted to take the most liberal view in all matters relating to the privileges offered by the Institute, a policy which I believe will tend to knit together in the closest bonds the men who are so unselfishly devoting their lives to the development of our science.

A number of men have availed themselves of the laboratory privileges during the year and we are glad to say that there is always room and the necessary supplies for the man who has a problem to solve and knows how to solve it.

At their recent meeting the Board of Managers of the Institute took a number of important steps for the promotion of our work. They authorized the Director to dispose of such materials of the museum as have only taxonomic interest and secure in lieu thereof materials related more properly to the problem of the Institute; they also authorized the support of a research room at the Woods Hole Laboratory, and a subvention to the American Journal of Anatomy. The Director was also authorized to make such arrangements with the Graduate School of the University of Pennsylvania for the promotion of research work in anatomy as will be mutually beneficial, and the same arrangements and privileges are to be extended to other universities which may desire to cooperate. The details of such arrangements will be considered by the Advisory Board.

M. J. Greenman.